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Multi-modality imaging assessment of cerebral small vessel disease biomarkers after stroke due to spontaneous intracerebral haemorrhage

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Declaration

I confirm that I composed this thesis and that it is my own original work except where explicitly stated otherwise in the text. I have not submitted this work for any other degree or professional qualification.

Chapter 7 of this thesis has been published in Rodrigues MA et al. The Edinburgh CT and genetic diagnostic criteria for lobar intracerebral haemorrhage associated with cerebral amyloid angiopathy: model development and diagnostic test accuracy study. (2018) The Lancet. Neurology. 17 (3): 232-240.

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Abstract

In most cases, spontaneous intracerebral haemorrhage (ICH) is thought to result from cerebral small vessel diseases (SVDs). Cerebral amyloid angiopathy (CAA) and arteriolosclerosis (non-CAA SVD) are the two main types of SVD associated with ICH. The risk of recurrent ICH and post-stroke dementia may be higher with CAA-associated ICH compared with non-CAA SVD-associated ICH. It is, therefore, important to identify the type of SVD associated with ICH. Commonly, ICH in the cerebral lobes (lobar ICH) is thought to be associated with CAA, whereas non-lobar ICH is associated with non-CAA SVD. However, this generalisation is not accurate.

The aims of my thesis were to (a) histopathologically assess the types and severity of SVDs associated with lobar and non-lobar ICH using brain autopsy tissue, (b) explore whether brain imaging (magnetic resonance imaging (MRI), positron emission tomography (PET) and computed tomography (CT)) biomarkers can be used in living patients to identify the type of SVDs associated with ICH and (c) determine whether CT biomarkers can predict outcome after ICH.

Throughout my thesis, I use data from two overlapping studies of ICH. The Lothian Audit of the Treatment of Cerebral Haemorrhage (LATCH) is a prospective community-based audit of all residents in the Lothian Health board region of Scotland who were aged 16 years or above and had an incident ICH between 1st June 2010 and 31st May 2013 inclusive. During LATCH and until 31st May 2016, consecutive adults with SVD-associated ICH were able to consent to have apolipoprotein E genotyping, brain MRI and brain autopsy as part of the prospective Lothian INtraCerebral Haemorrhage Pathology, Imaging and Neurological outcome (LINCHPIN) study.

In an autopsy study of 126 LINCHPIN participants with first-ever SVD-associated ICH, I found that 98% of participants with a non-lobar ICH had moderate or severe non-CAA SVD. In contrast, 60% of participants with a

lobar ICH had moderate or severe CAA, while many had moderate or severe non-CAA SVD, either with or without CAA.

The most common approach used in clinical practice to identify CAA-associated ICH is the MRI-based modified Boston criteria for CAA. However, I found that only about one-third of ICH patients during LATCH were able to undergo MRI. Also, in 16 LINCHPIN participants with research MRI, the modified Boston criteria showed limited accuracy against the histopathological assessment of CAA on subsequent research autopsy.

Molecular imaging using tracers designed to detect parenchymal β-amyloid in Alzheimer's disease, such as flutemetamol, may also identify the perivascular β-amyloid found in CAA. I found that in LINCHPIN brain tissue samples, the amyloid tracer flutemetamol labelled both parenchymal and perivascular β-amyloid. Among 20 participants with first-ever ICH, flutemetamol PET scans were 86% sensitive and 77% specific for CAA-associated ICH based on the modified Boston criteria.

Brain CT is the test that usually diagnoses ICH. I found in 62 LINCHPIN participants with first-ever lobar ICH that subarachnoid haemorrhage and finger-like projections on CT, and APOE ε4 allele possession were independently associated with CAA-associated lobar ICH defined on subsequent autopsy. I developed diagnostic models and criteria ("Edinburgh criteria") based on these predictors that could accurately rule in or exclude CAA-associated lobar ICH. I performed a multicentre external validation study of the Edinburgh criteria. In a preliminary analysis, the CT-only diagnostic criteria (subarachnoid haemorrhage and finger-like projections) had high sensitivity (88%) and good specificity (84%) for CAA-associated lobar ICH.

The risk of recurrent ICH is a key outcome for ICH survivors. I found that the risk of recurrent ICH was significantly higher in participants with first-ever lobar compared with non-lobar ICH. Among lobar ICH participants, the risk of recurrent ICH was significantly higher in those classified as high risk on the

CT-only Edinburgh criteria for CAA-associated lobar ICH compared with those classified as low risk.

Predicting death or disability is also important in ICH. I found that the severity of SVD on the diagnostic brain CT was an independent predictor of death or disability at one year after the ICH, after adjusting for known prognostic factors.

Brain imaging biomarkers, particularly on brain CT, are potentially useful for identifying the type of SVD associated with ICH. Having developed the CT-based Edinburgh criteria for CAA-associated lobar ICH, I performed preliminary multicentre studies assessing their diagnostic accuracy and prognostic value. In the future, I aim to perform the full analyses of these diagnostic test accuracy and prognostic studies to determine the clinical utility of the Edinburgh criteria. Other research should assess the clinical and economic effect of the Edinburgh criteria on the prognosis of ICH. More work is needed to investigate the prognostic relevance of coexistence of CAA and non-CAA SVDs in lobar ICH, and whether these groups can be differentiated using imaging biomarkers.

Lay summary

Stroke due to bleeding within the brain is called intracerebral haemorrhage (ICH). In most cases, ICH is thought to caused by bleeding from previously damaged small blood vessels in the brain (small vessel diseases – SVD). SVD can result from an abnormal protein accumulating in the small blood vessel walls, known as cerebral amyloid angiopathy (CAA), or high blood pressure (non-CAA SVD). Identifying ICHs associated with CAA is important because the risk of recurrent ICH appears higher in these patients.

The aims of my thesis were to (a) assess the types and severity of SVD associated with ICH using brain autopsy tissue, (b) explore whether brain imaging (magnetic resonance imaging (MRI), molecular imaging and computed tomography (CT)) features can be used in living patients to identify CAA-associated ICH and (c) determine whether brain CT features can predict outcome after ICH.

In an autopsy study of 126 ICH participants, I found that almost all with an ICH deep within the brain (non-lobar ICH) had advanced non-CAA SVD. In contrast, 60% of those with a bleed located superficially in the brain (lobar ICH) had advanced CAA, while many had advanced non-CAA SVD, either with or without CAA.

Identifying lobar ICH patients who have CAA is useful for predicting outcome. The most common approach used in clinical practice is brain imaging with MRI. However, I found that only about one-third of ICH patients are able to undergo MRI. Also, the widely used MRI criteria for CAA showed limited accuracy for CAA identified at subsequent autopsy, which is the best approach for identifying CAA.

Molecular brain imaging using tracers designed to detect the amyloid protein in Alzheimer's disease may also detect the amyloid protein found in CAA. I found that in brain tissue samples, the amyloid tracer labelled both Alzheimer's and CAA amyloid. Molecular amyloid scans showed good accuracy for CAA in ICH patients based on the MRI criteria.

Brain CT is the test that usually diagnoses ICH. I found that two features on the diagnostic brain CT (blood in the fluid around the brain and a lobulated contour of the ICH) were highly accurate for identifying CAA-associated lobar ICH at subsequent autopsy. CT is widely available, so these diagnostic criteria for CAA-associated lobar ICH are potentially useful worldwide.

One of the main concerns in ICH survivors is their risk of a recurrent ICH. I found that the CT criteria for CAA-associated ICH were able to identify lobar ICH patients at high or low risk of recurrent ICH.

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My thesis is centred around two overlapping research studies – the LATCH and LINCHPIN studies – which were conducted by members of the RUSH research team at the University of Edinburgh. In particular, I would like to thank Dr Neshika Samarasekera and Professor Rustam Al-Shahi Salman for designing the studies, and the entire RUSH team for rigorously identifying participants and collecting data. I am grateful to Aidan Hutchison, IT programmer, Rosemary Anderson, research administrator, and Christine Lerpiniere, senior research nurse, for helping orientate me to the study databases, assisting in database queries and providing general life advice. Professor Colin Smith was integral to the LINCHPIN study, performing all of the research brain autopsies. Dr Catherine Humphreys also helped rate the histopathological samples. Katie McGoohan, Research Nurse, helped prepare the regulatory approvals for the flutemetamol MR-PET study. Dr Gerry Thompson helped rate the flutemetamol PET scans, and Dr Giorgos Papanastasiou helped me develop the quantitative analysis pipeline. Karina McDade, Senior Research Technician, and Dr Juraj Koudelka, research fellow, helped me perform the *ex vivo* 6-CN-flutemetamol study.

I was fortunate to work with many researchers from Europe and North America to perform a multicentre external validation study of the Edinburgh Criteria for CAA-associated lobar ICH. I am hugely grateful to my collaborators who provided comprehensive clinical, imaging and pathological data in a short time frame. In particular, I would like to thank Dr Andreas Charidimou, Harvard Medical School, for his assistance in setting up and performing this study, and Dr David Seiffge for analysing the data from the CROMIS-2 study.

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Publications arising from work associated with this thesis

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Section A. Introduction

Chapter 1 Introduction

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1.1 Cerebral small vessel diseases

Cerebral small vessel diseases (SVDs) are pathological processes affecting the small blood vessels in the brain.[1] Small blood vessels are defined as vascular structures in the brain parenchyma or subarachnoid space (leptomeningeal) ranging from 2 mm to 5 μm in diameter and include small arteries, arterioles, capillaries and venules.[1] The term SVDs is often used to relate to the small arteries and arterioles. However, it also encompasses diseases of the venules and capillaries.[2, 3]

Anatomically, there are two origins of the cerebral small arteries and arterioles. They can arise directly from large arteries at the base of the brain as deep arterial perforators and supply the basal ganglia (lenticulostriate and anterior choroidal arteries), thalami (predominantly posterior choroidal and thalamogeniculate arteries) and brainstem (predominantly small perforating branches of the basilar and superior cerebellar arteries). Alternatively, they can originate from medium-sized arteries in the subarachnoid space on the brain surface and penetrate the cortex superficially to supply the grey-matter and subcortical white matter. These two systems converge in the deep subcortical white matter, although they do not anastomose.[1, 4-7]

1.1.1 Types of SVDs

There are several categories of underlying pathology in SVDs, of which arteriolosclerosis and cerebral amyloid angiopathy (CAA) are the most common. Genetic SVDs, such as cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and Fabry's disease, are a less frequent type of SVD.[8-11] Inflammatory and immunologically mediated SVDs, such as granulomatosis related to polyangiitis, Churg-Strauss syndrome and systemic vasculitis associated with connective tissue disorders,[12] venous collagenosis and post-radiation

angiopathy can affect cerebral small blood vessels but are not typically considered under the term SVDs.

In my thesis, I will concentrate on SVDs thought to result from arteriolosclerosis and sporadic CAA, and I will use the term SVDs to refer to these two types of SVD.

1.1.1.1 Arteriolosclerosis

Arteriosclerosis is common, affecting up to 70% of the elderly.[14] It is associated with hypertension, ageing and diabetes,[2, 15, 16] although many people with arteriolosclerosis are not hypertensive.[2, 17] It has variably been called arteriolosclerosis, age-related or vascular risk-factor-related SVD, hypertensive arteriopathy or sporadic non-amyloid microangiopathy in the literature.[1, 4]

Arteriolosclerosis is characterised by the initial hypertrophy then subsequent loss of smooth muscle cells in the media of small vessels (cerebral, retinal, renal), collagenous thickening of the vessel wall and deposition of fibro-hyaline material. It typically affects the deep arterial perforators supplying the basal ganglia, thalami and brainstem.[1]

The pathophysiology of arteriolosclerosis is not fully understood. At a simple level, the pathological changes can be thought to result in luminal narrowing and dilatation, microatheroma and microaneurysms, which in turn can cause ischaemic or haemorrhagic (micro and macrohaemorrhages) consequences. However, the vascular changes involved are much more complicated, with ischaemia and haemorrhages being end-stage events of a long-standing complex cascade of vascular dysfunction.

1.1.1.2 Sporadic CAA

CAA can occur as a sporadic form or as rare inherited versions.[18] Sporadic CAA frequently occurs in the general elderly population, affecting 20-40% of non-demented elderly and 50-60% of demented elderly,[14, 19] and in Alzheimer's disease.[14, 19-21] Increasing age is the strongest clinical risk factor for CAA.[22, 23] The apolipoprotein E (APOE) ε4 allele has a dose-

dependent association with pathologically proven sporadic CAA,[24, 25] whereas a recent meta-analysis did not show a statistically significant association between APOE ε2 allele and sporadic CAA.[24]

CAA is characterised by progressive accumulation of perivascular β-amyloid peptide in the media and adventitia of cortical and leptomeningeal arteries and arterioles.[26] The cerebral cortex is most commonly affected. However, the cerebellum can also be involved to a lesser extent.[23, 27, 28] β-amyloid in CAA is predominantly composed of the 40 amino acid fragment, whereas the 42 amino acid fragment is the major type in Alzheimer's disease.[29-31] The pathophysiology of vascular β-amyloid deposition is incompletely understood. Current theories suggest it results from impaired clearance rather than overproduction of the β-amyloid protein,[32, 33] via disrupted perivascular drainage pathways.[34, 35]

When severe, CAA can result in the complete replacement of vascular smooth muscle with β-amyloid, and the vessels become irregular, dilated and demonstrate secondary vasculopathic changes, such as fibrinoid necrosis and microaneurysm formation.[33, 36] Vessel disruption and fragmentation is thought to result in extravasation of blood (micro and macrohaemorrhages),[1] while luminal occlusion can result in ischaemia and infarction.[37]

1.1.2 Clinical consequences of SVDs

SVDs can result in both ischaemic and haemorrhagic consequences. SVDs cause 20-25% of ischaemic strokes[38, 39] and 85% of spontaneous intracerebral haemorrhage,[40] yet the pathophysiology is incompletely understood.[1]

As described above, the pathophysiology underlying arteriolosclerosis and CAA is not fully understood. Several mechanisms have been suggested for ischaemic consequences of SVDs. SVD-related vessel wall thickening can cause a narrowing of the vessel lumen. This is postulated to result in chronic white matter hypoperfusion, leading to oligodendrocyte death and ultimately

demyelination.[41-43] Endothelial dysfunction, disruption of the blood-brain barrier and oligodendrocyte apoptosis may also contribute to diffuse chronic white matter interstitial fluid increase with or without hypoperfusion, resulting in white matter lesions on neuroimaging (1.1.3).[2, 3, 44-46] Endothelial dysfunction and inflammation may precipitate acute occlusion of the vessel lumen and is considered to be the cause of most small subcortical infarcts, while a minority of acute small subcortical infarcts are thought to result from large vessel atheroma and embolism.[2, 44, 47-50]

The haemorrhagic consequences are hypothesised to relate to SVD-related vessel wall damage, which can lead to fragile, brittle walls and microaneurysm formation, especially in CAA. Subsequent vessel rupture is thought to account for microscopic (cerebral microbleed (CMB) – see 1.1.3.5) and macroscopic haemorrhages.

SVDs are a major contributor to cognitive impairment in the elderly,[51] accounting for nearly half of dementias.[52] Strategically located lacunes,[53] particularly in the thalamus,[54] as well as multiple lacunes[55] are associated with worse cognition. White matter lesions thought to be related to SVDs are associated with cognitive decline in longitudinal studies,[56] and act as a predictor for cognitive decline[57] and dementia.[58] CAA is an important contributor to cognitive decline independent of Alzheimer's disease and other dementia-related neuropathologies.[14, 21, 59, 60] The mechanisms through which CAA causes cognitive impairment are not understood but may relate to CMBs, microinfarcts and white matter lesions.[61-63]

The presence and severity of SVDs are associated with the development of physical disabilities in the elderly.[64, 65] Patients with severe white matter lesions have a twofold higher risk of developing a disability or dying compared to patients with mild white matter lesions.[65]

CAA can also result in transient focal neurological episodes ("amyloid spells").[66] These episodes commonly involve spreading sensory symptoms, such as paraesthesia or numbness, although motor seizure-like

episodes and visual disturbance can also occur. They usually progress over seconds to minutes, last less than 30 minutes and are often recurrent and stereotyped.[23, 66] Their cause is unclear; they may relate to a direct effect of β-amyloid on local cortical function, or reflect seizure-like activity from local haemorrhage,[67, 68] spreading cortical depression[69, 70] or local vasospasm.[23, 66, 69] CAA-associated transient focal neurological episodes may be associated with an increased risk of early symptomatic lobar ICH.[71]

1.1.3 Neuroimaging features of SVDs

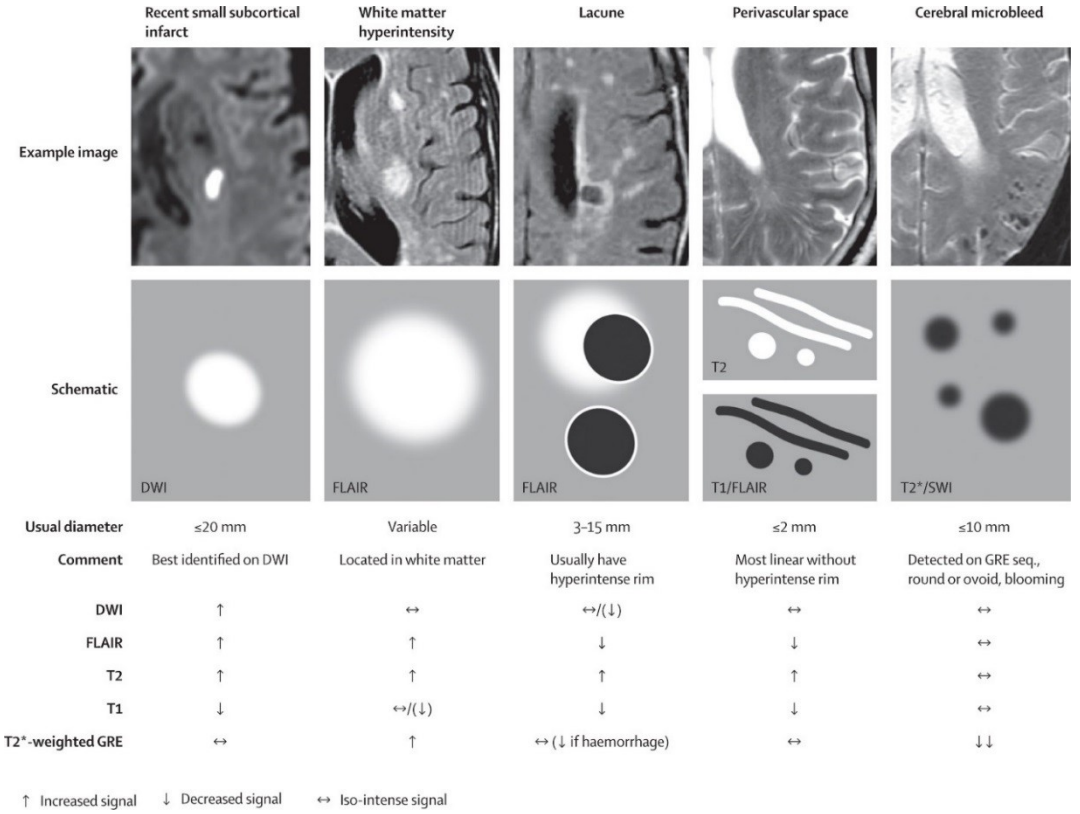
Studying SVDs is challenging. There is often limited tissue available for histopathological assessment as brain biopsies are invasive and seldom performed, while post-mortem material is scant as many SVDs are rarely fatal in the acute setting.[72, 73] Animal models are currently used with caution because of lack of a clear mechanism to mimic.[74, 75]

The small cerebral vessels affected by SVDs are generally too small to directly identify on clinical neuroimaging.[76] However, neuroimaging can identify brain changes and vascular dysfunction thought to represent some of the consequences of SVDs, making it a useful technique for studying SVDs. Magnetic resonance imaging (MRI) is the neuroimaging reference standard for SVDs because it has better sensitivity and specificity for SVD imaging biomarkers compared with computed tomography (CT).[77] However, CT is more widely available and better tolerated than MRI, making it a useful imaging modality in routine clinical practice.

The nomenclature and definitions of SVD neuroimaging biomarkers has varied considerably in the past.[77-79] In 2013, an international working group published a position paper proposing a unified approach to defining and reporting SVD neuroimaging biomarkers (Standards for ReportIng Vascular changes on nEuroimaging [STRIVE], Figure 1.1), which is now widely accepted.[77]

Figure 1.1 STRIVE definitions of MRI SVD biomarkers

Example axial MRI images (upper) and schematic representations (middle) of SVD MRI biomarkers, with a summary of imaging characteristics (lower) for individual lesions. Reproduced with permission from [77]



DWI = diffusion-weighted imaging. FLAIR = fluid-attenuated inversion recovery. GRE = gradient-recalled echo. MRI = magnetic resonance imaging. STRIVE = standards for reporting vascular changes on neuroimaging. SWI = susceptibility-weighted imaging.

1.1.3.1 Recent small subcortical infarcts

A recent small subcortical infarct is an area of infarction in the territory of a perforating arteriole (i.e. subcortical white matter, basal ganglia, thalami or brainstem) with neuroimaging features or clinical symptoms consistent with a lesion occurring in the preceding few weeks.[77] It should be less than 20 mm in axial diameter. There is no minimum diameter.

Recent small subcortical infarcts usually correspond with a clinically evident lacunar stroke syndrome, although occasionally they can be asymptomatic.[80, 81] MRI, however, is not completely sensitive for acute lacunar stroke syndrome as there is no corresponding recent small subcortical infarct in up to 30% of patients.[82] Evolution of small subcortical infarcts is variable; they may become a lacune or white matter hyperintensity, or disappear.[77]

1.1.3.2 Lacune of presumed vascular origin

A lacune of presumed vascular origin (lacune) is a small round or ovoid subcortical cavity consistent with a previous acute brain infarct or haemorrhage in the territory of a perforating arteriole.[77] Lacunes range in size from 3 to 15 mm. The contents of the cavity usually follow the signal of cerebrospinal fluid (CSF), however, sometimes the central cavity can have high signal on FLAIR.[83] There is often a rim of hyperintense FLAIR signal. On CT, lacunes are small focal areas of subcortical hypoattenuation. Most lacunes are thought to be the sequelae of symptomatic or asymptomatic small subcortical infarcts, although some may evolve from small subcortical haemorrhages.[77, 84] Post-mortem MRI studies show that MRI-defined lacunes correlate with irregular cavities, around which there may be reactive gliosis with myelin and axonal loss.[73] Some lacunes do not show cavitation but are associated with selective neuronal loss.[73]

Lacunes are common in asymptomatic elderly patients, but they are also associated with an increased risk of stroke, dementia and gait impairment.[85-88]

1.1.3.3 White matter hyperintensity of presumed vascular origin

White matter hyperintensities of presumed vascular origin (WMH) are areas of increased T2-weighted signal.[77] T2-weighted hyperintensities in the deep grey matter and brainstem are not usually included in this definition. WMH are usually bilateral and often symmetrical. Periventricular WMH are often differentiated from subcortical or deep WMH.[89] The terms white matter hypoattenuation and white matter lucencies are used for describing these abnormalities on CT.

WMH are correlated with a heterogeneous variety of histopathological findings, including myelin, axonal and oligodendrocyte loss, dilated perivascular spaces, white matter infarcts and arteriolosclerosis.[73] The severity of histopathological changes mirrors the severity of WMH on neuroimaging; smooth periventricular or punctate deep WMH are associated with mild changes on histopathology, whereas irregular periventricular and confluent deep WMH correlate with more severe histopathological features.[73]

WMH are associated with cerebrovascular disease and traditional vascular risk factors, as well as an increased risk of stroke, dementia and death.[90] Their pathogenesis is not fully understood but may relate to chronic hypoperfusion, endothelial dysfunction, disruption of the blood-brain barrier and oligodendrocyte apoptosis (1.1.2).

1.1.3.4 Perivascular spaces

Perivascular spaces are fluid-filled spaces following the expected course of a perforating vessel in grey or white matter and have the same signal intensity as CSF.[77] They are linear when imaged parallel to the vessel and round or ovoid and less than 3 mm in diameter when imaged perpendicular to the vessel. Perivascular spaces are seen in the basal ganglia, cerebral white matter and midbrain. They usually do not have a hyperintense T2-weighted rim, unlike lacunes, unless they occur in a WMH.[73, 91]

Normal perivascular spaces are microscopic and not visible on MRI. Only when they enlarge do they become visible. They are associated with other

SVD neuroimaging biomarkers, such as WMH[92] and lacunes.[93] They are also postulated to be involved in the pathogenesis of CAA (1.1.1.2).[94, 95]

1.1.3.5 Cerebral microbleeds

A CMB is a small area of signal loss between 2 mm and 10 mm in diameter with associated blooming on T2*-weighted or susceptibility-weighted sequences.[77] They are usually round or oval with homogeneously low signal on T2*-weighted or susceptibility-weighted imaging, and not visible on T1-weighted, T2-weighted or FLAIR sequences. CMBs are most frequently seen at the cortico-subcortical junction, deep cerebral or cerebellar grey and white matter, and in the brainstem.

CMBs have been shown to correlate with tiny foci of hemosiderin on histopathology. However, some CMBs relate to small lacunes, vessel wall dissection and microaneurysms.[73, 96, 97] CMBs are associated with SVDs.[98-100] The adjacent small blood vessels are often abnormal, being affected by arteriolosclerosis or CAA.[101, 102] CAA-associated CMBs tend to be located at the cerebral grey-white matter junction,[103] whereas CMBs associated with arteriolosclerosis tend to be found in the non-lobar regions.[104] CMBs restricted to the lobar regions is a key imaging feature of CAA-associated lobar ICH and are associated with an increased risk of recurrent ICH in such patients.[105] They are also associated with cognitive decline.[106] However, the strengths of associations and specificity of CMB distribution remain unclear.

1.1.3.6 Cortical superficial siderosis

Cortical superficial siderosis is defined as curvilinear hypointensities over the cortex with blooming artefact on T2*-weighted or susceptibility-weighted imaging.[77, 107, 108] The hypointense signal should run parallel to the cerebral sulci and superficial cortical layers. The sensitivity of MRI for cortical superficial siderosis depends on the sequence type and field strength.[107, 109]

Cortical superficial siderosis relates to haemosiderin deposits within the subarachnoid space, the leptomeninges and the superficial (subpial) layers of

the cerebral cortex.[107] It is thought to result from the breakdown of acute cerebral convexity subarachnoid haemorrhage.[108, 110-112]

Cortical superficial siderosis can result from traumatic subarachnoid haemorrhage, vascular malformations, reversible cerebral vasoconstriction syndrome, or it can be idiopathic.[107, 113, 114] It is now recognised as an important imaging biomarker of CAA.[107, 110, 115] In CAA, cortical superficial siderosis is thought to be a marker of cortical or leptomeningeal vessel rupture or expansion of a lobar ICH into the subarachnoid space. It is associated with transient focal neurological episodes [71] and an increased risk of recurrent spontaneous ICH.[116-118]

1.1.3.7 Brain atrophy

Brain atrophy is defined as a reduced volume of brain parenchyma that is not related to a specific focal macroscopic injury, such as previous trauma or an infarct.[77] Its presence is inferred by enlargement of the CSF containing spaces; cortical sulci for cortical atrophy and the ventricular system for central atrophy.

Brain atrophy can be generalised or focal, symmetrical or asymmetric. It occurs in many neurological disorders as well as normal ageing. Brain atrophy related to SVDs is a heterogeneous process and corresponds to neuronal loss, white matter rarefaction and secondary neurodegenerative changes.[119-121]

SVD severity is associated with brain atrophy.[122, 123] There is also evidence that CAA is associated with brain atrophy even in the absence of Alzheimer's disease.[124, 125] Brain atrophy is thought to account for at least some of the cognitive impairment associated with SVDs.[126, 127]

1.2 Symptomatic spontaneous ICH

1.2.1 Terminology

Spontaneous ICH refers to bleeding within the brain parenchyma, which is not caused by trauma.[128] Spontaneous ICH can extend into the extra-axial

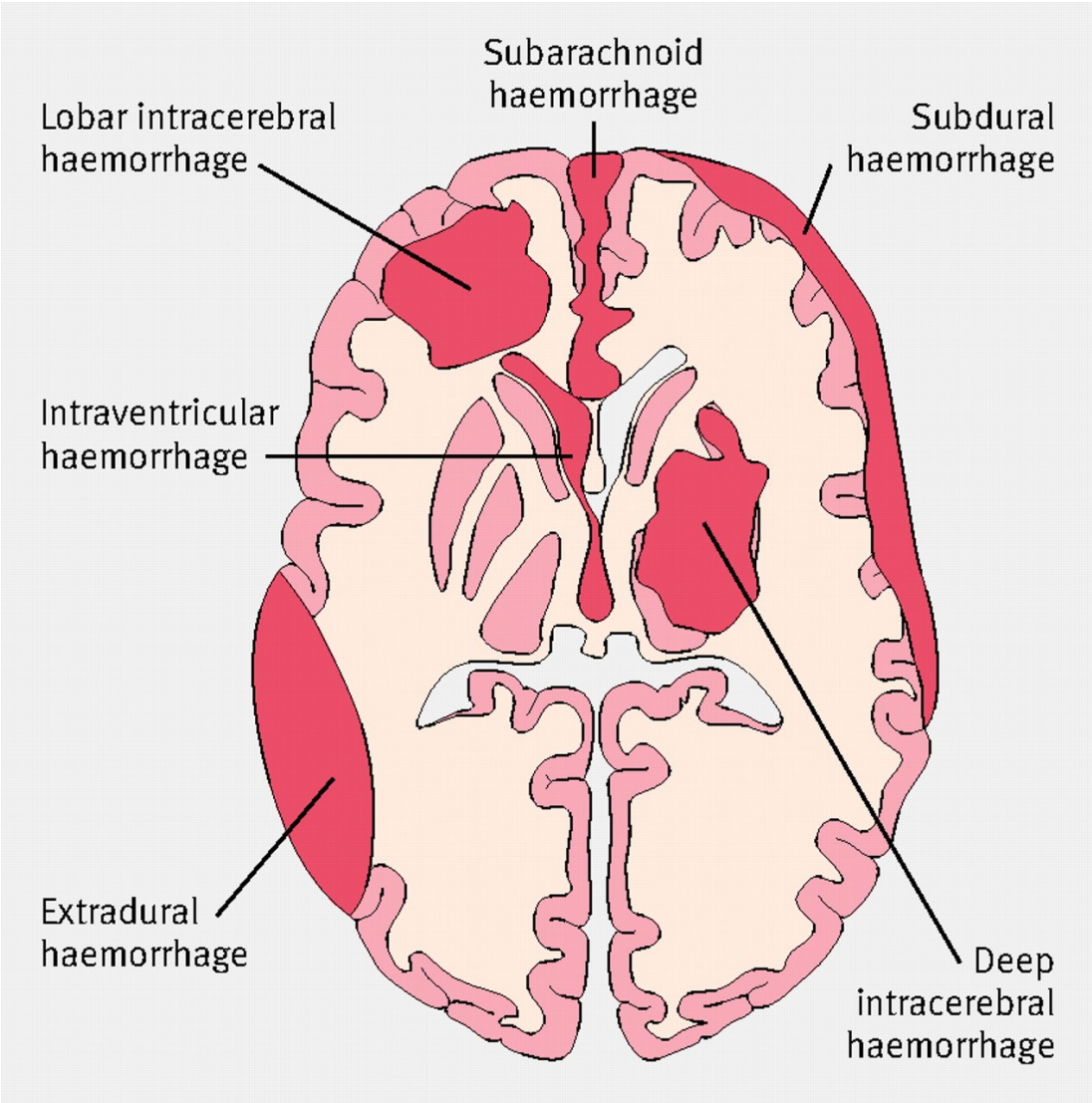
spaces (intraventricular, subarachnoid or subdural). However, it is important to distinguish spontaneous ICH from pure intraventricular, subarachnoid, subdural or extradural haemorrhage (Figure 1.2) given the differences in risk factors, aetiology and treatments.[129] Spontaneous ICH and subarachnoid haemorrhage are sometimes considered together under the term "haemorrhagic stroke".

Spontaneous ICH can be further classified based on the underlying aetiology (Section 1.2.6). "Primary ICH" is often used in the literature and clinical practice to refer to an ICH where no macroscopic cause is evident and the ICH is presumed due to SVDs. However, this term is confusing, as it implies there is no cause underlying the ICH. "Secondary ICH" is used when a macroscopic cause, such as an aneurysm, arteriovenous malformation or tumour, is present.

Throughout my thesis, I use the abbreviation ICH to represent spontaneous ICH, the term haemorrhagic stroke to represent ICH and subarachnoid haemorrhage, and SVD-associated ICH to represent spontaneous ICH presumed secondary to SVDs (i.e. when no macroscopic cause of ICH is evident).

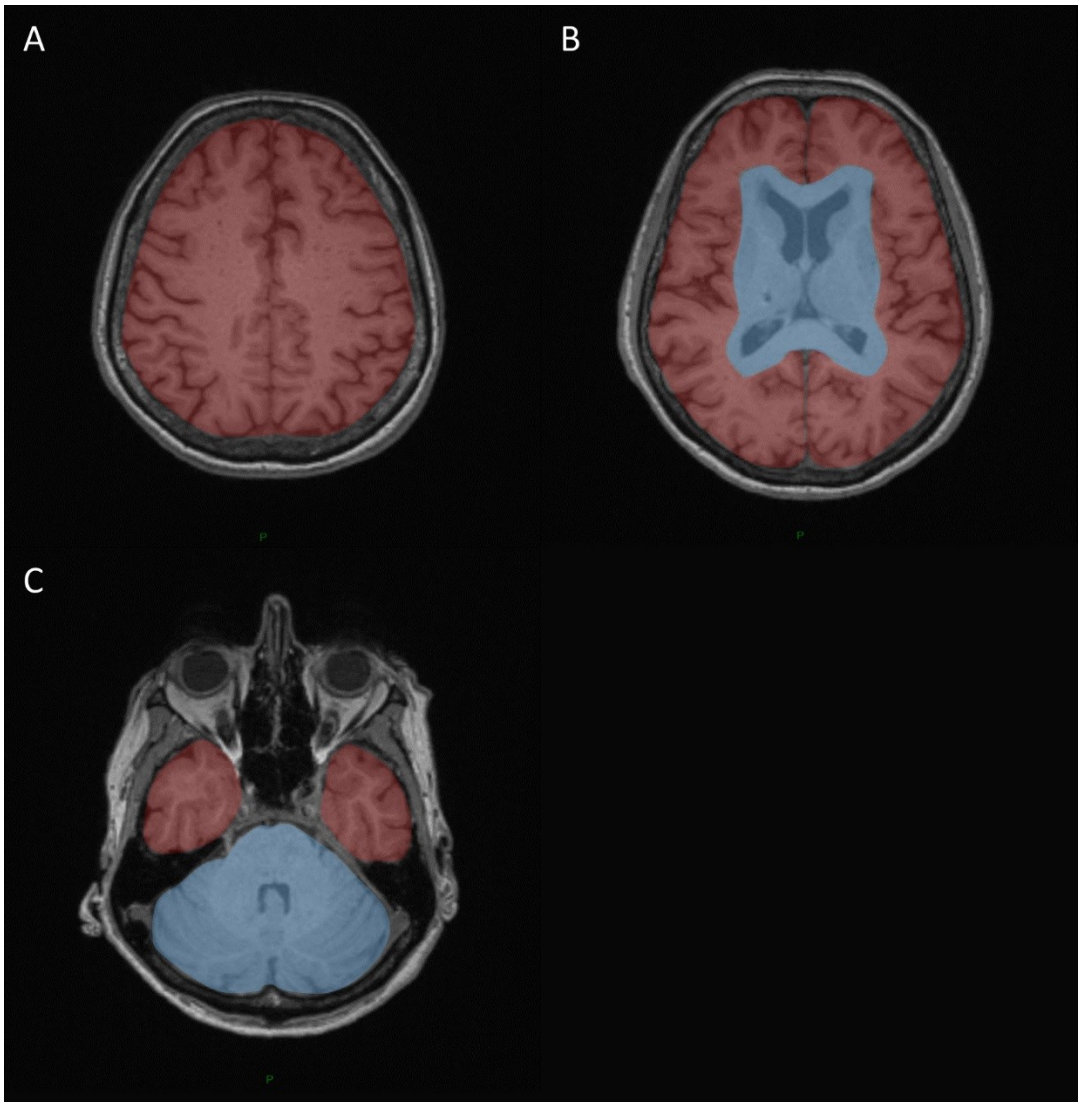
Figure 1.2 Axial illustration of the brain showing the subtypes of intracranial haemorrhage.

Reproduced with permission from **[129]**



In research and clinical practice, SVD-associated ICH is frequently categorised by anatomical location because this partly influences the likely underlying SVDs (1.1.1) and prognosis (1.2.7). In my thesis I use the term lobar ICH to refer to haematomas where the main bulk and presumed epicentre is in the cerebral lobes (frontal, parietal, temporal or occipital lobes, or the insular cortex). I use the term non-lobar ICH when the main bulk and presumed epicentre of the haematoma is in the basal ganglia, thalamus, brainstem or cerebellum (Figure 1.3).

Figure 1.3 Axial T1-weighted images of the brain showing the lobar (red shaded) and non-lobar (blue shaded) regions



1.2.2 The burden of ICH

Stroke is the second most common cause of death worldwide and the second most common cause of disability.[130] Haemorrhagic stroke accounts for approximately one-third of incident strokes worldwide, but 49% of stroke deaths and 58% of disability-adjusted life years due to stroke.[131] Haemorrhagic stroke is a global disease. However, its main burden is in low and middle-income countries; 80% of incident haemorrhagic strokes, 84% of deaths from haemorrhagic strokes and 86% of disability-adjusted life years

due to haemorrhagic stroke occurred in low and middle-income countries.[132]

ICH is the major cause of haemorrhagic stroke and the second most common cause of stroke after ischaemic stroke. Between 2000 and 2008, ICH accounted for 9% to 13% of strokes in high-income countries, and 14% to 27% in low to middle-income countries.[133]

1.2.3 Epidemiology of ICH

The overall incidence of ICH in 29 population-based studies without age limits was 23.5 per 100,000 person-years (95%CI 20.1-27.6).[134] The incidence of ICH in low to middle-income countries was nearly double the incidence in high-income countries. The difference in ICH incidence between high-income and low to middle-income countries is thought to relate to differences in the frequency of risk factors, such as hypertension and smoking.[133]

The incidence of ICH increases with age and is highest amongst east and southeast Asian people compared with other ethnicities (incidence ratio 2.1, 95%CI 1.6-2.9 compared with white people).[134] The incidence in women is lower than men, although this difference is not statistically significant (incidence ratio 0.85, 95%CI 0.61-1.18).[134]

Overall there has been no substantial decrease in the incidence of ICH since 1980 in 26 high-quality population-based studies (annual decrease of 0.3% per year, 95%CI -2.7-3.3).[134] In one population-based study from Oxford, the change in ICH incidence over time varied according to the presumed aetiology of ICH. The incidence of ICH associated with premorbid hypertension fell between 1981 and 2006 (rate ratio 0.37, 95%CI 0.20-0.69), whereas the incidence of antithrombotic-associated ICH (rate ratio 7.4, 95%CI 1.7-32) and ICH associated with presumed CAA (rate ratio 4.0, 95%CI 1.1-17) increased.[135] Similar findings were identified between 1985 and 2008 in a population-based registry of ICH in Dijon, France.[136] There is no accurate data on the change in stroke incidence according to stroke

subtype in low and middle-income countries before 2000, preventing the assessment of temporal changes in ICH incidence in these countries.[133]

1.2.4 Risk factors for spontaneous ICH

Hypertension is the most commonly identified risk factor for ICH. The risk of ICH in those with hypertension is two times that of non-hypertensive people.[137-140] The risk also appears to increase with increasing average blood pressure,[137] and is higher for non-lobar compared with lobar ICH.[138, 141] Increasing age is another strong risk factor for ICH, with the relative risk of ICH doubling with every decade increase.[137, 139] High alcohol intake is also associated with an increased risk of ICH.[137, 140, 142] Genetic polymorphisms account for up to 44% of ICH risk.[143] APOE is the best-studied gene. The presence of APOE ε2 and ε4 alleles are associated with ICH, especially lobar ICH.[138, 144, 145] However, APOE only accounts for a proportion of the genetic risk for ICH, and other genes are also likely to influence the risk of ICH.

1.2.5 Clinical presentation of spontaneous ICH

The clinical features caused by ICH depend on its size and location. They can relate to damage in the area affected by the ICH, remote effects of displaced brain, as well as raised intracranial pressure. ICH usually results in an abrupt onset of clinical features. Patients usually have an acute stroke syndrome with focal neurological deficit. They may have a severe headache, nausea, vomiting and altered consciousness. Patients may also present with confusion, personality change or seizures. Some ICH can be asymptomatic.[146]

Currently, it is not possible to reliably differentiate ICH from ischaemic stroke clinically.[147] Neuroimaging is required to confirm ICH.[148, 149]

1.2.6 Cause of spontaneous ICH

Spontaneous ICH is caused by a specific macroscopic structural cause, such as an arterial aneurysm, arteriovenous malformation, cerebral cavernous malformation, venous sinus thrombosis, tumour or abscess, in approximately

15% of cases.[40] It is important to identify such patients as there may be specific management options depending on the underlying cause.[150]

The majority of patients with spontaneous ICH, however, have no macroscopic structural cause. In these patients, the ICH is thought to be due to SVDs, either arteriolosclerosis or CAA (SVD-associated ICH).[40] As discussed in 1.1.2, SVD-related vessel wall damage is thought to lead to vessel rupture and ICH.

1.2.7 Prognosis of SVD-associated ICH

The most clinically relevant outcomes in SVD-associated ICH are death, disability and recurrent ICH.

1.2.7.1 Death

Median one-month case fatality for ICH is approximately 40%.[133, 134] Median one-year case fatality is approximately 55%.[134] Overall, case fatality increases with age and is similar for men and women. There has been no substantial change in one-month case fatality of ICH since 1980 (annual decrease 0.2% per year, 95%CI -1.1 to 0.7).[134]

The most well-established risk factors for death after ICH are increasing age, increasing ICH volume, decreasing Glasgow Coma Scale (GCS) score on hospital admission and infratentorial ICH location.[151]

1.2.7.2 Disability

Survivors of SVD-associated ICH are often left disabled. A systematic review of four population-based studies of functional outcome after ICH demonstrated that 16-46% of survivors were functionally dependent at six months (modified Rankin scale 3-5), while 43-46% were dependent at one year.[151] Predictors of functional outcome after ICH are similar to the risk factors for death. Pre-ICH cognitive impairment is also independently associated with functional outcome.[152]

The severity of SVDs may be a risk factor for death and disability after SVD-associated ICH given its association with physical disabilities and death in elderly patients without ICH.[64, 65]

1.2.7.3 Recurrent ICH

The risk of a recurrent ICH is important for survivors of SVD-associated ICH. The annualised rate of recurrent ICH is 2.0-2.4%, with 1.8% to 7.4% of patients having a recurrent ICH within the first year.[151] Patients with a lobar ICH appear to be at higher risk than non-lobar ICH.[151] This is thought to reflect the likely types of SVDs underlying these ICHs. CAA is associated with some lobar ICH,[153] whereas non-lobar ICH is usually associated with arteriolosclerosis. MRI-based studies suggest the risk of recurrent ICH is higher with CAA-associated ICH rather than arteriolosclerosis-associated ICH.[105, 154-157]

The risk of antithrombotic-related recurrent ICH may also be higher in survivors of CAA-associated lobar ICH. Biffi et al. assessed the association between clinical characteristics, APOE genotype, neuroimaging features and antithrombotic drug use and the risk of recurrent ICH in CAA-associated lobar ICH in a single centre prospective cohort study of 104 SVD-associated lobar ICH survivors.[158] A previous lobar ICH, two or more lobar CMBs, posterior white matter lucencies on CT and aspirin use after the index ICH were independently associated with the risk of recurrent ICH. However, the study had several limitations. Only survivors of lobar ICH who underwent a MRI were included, which is likely to introduce a selection bias. The sample size was modest, with only 29 recurrent ICHs during follow-up. The regression models were overfitted, especially given the stepwise selection methods used. The authors did not account for death as a competing risk, which will overestimate the strength of the reported associations. Finally, the findings may be the result of confounding as this was not a randomised study. In particular, the authors did not account for blood pressure control during follow-up, which is known to affect the risk of recurrent ICH.[159]

A recent randomised controlled trial assessed the risk of antiplatelet drugs on recurrent ICH in SVD-associated ICH survivors who were taking an antiplatelet drug for the prevention of occlusive vascular disease at the time of ICH. In contrast to the findings described by Biffi et al, in this study the group restarted on an antiplatelet agent after the ICH had a borderline

statistically significant lower risk of recurrent ICH than the group not restarted on an antiplatelet drug (adjusted hazard ratio 0.51, 95% CI 0.25–1.03; p=0.060).[160] There were no statistically significant hazards of antiplatelet drug use on recurrent ICH according to CT or MRI markers of CAA. However, the power of these imaging subgroup analyses was limited, and there is likely to be selection bias in the study.[161]

It is therefore unclear whether aspirin does increase the risk of recurrent ICH in those with a CAA-associated lobar ICH. Nonetheless, clinicians often avoid antithrombotic drugs in patients with a presumed CAA-associated ICH.

1.3 Diagnosing SVD-associated ICH

Differentiating CAA-associated ICH from arteriolosclerosis-associated ICH is clinically important to inform prognosis and potentially guide some treatment decisions. However, diagnosing the types of SVDs underlying SVD-associated ICH is currently difficult.

1.3.1 Histopathology

Histopathology is the reference standard for diagnosing SVD-associated ICH. Arteriolosclerosis and CAA have characteristic features on histology (1.1.1) and CAA can be accurately identified using immunohistochemistry. Furthermore, other causes of ICH, such as aneurysms, arteriovenous malformations and tumours, may be excluded. However, brain tissue is rarely available during life. A cortical biopsy is an invasive procedure, and haematoma evacuation samples are not commonly available given the lack of clear benefit from early surgical haematoma evacuation.[162, 163] Even when tissue is available for histopathology, the quantity is often small, and inaccuracies can occur due to sampling error.[164] Therefore, differentiation of CAA-associated and arteriolosclerosis-associated ICH is usually based on ICH location and neuroimaging features.

1.3.2 ICH location

Given the distribution of vessels affected,[1, 26, 28] clinicians frequently attribute non-lobar ICH to arteriosclerosis and lobar ICH, particularly in the elderly, to CAA.[165] However, these generalisations are not completely accurate. The link between CAA and lobar ICH is relatively weak, being based on a few clinicopathological case-control studies with methodological problems, such as poorly matched control groups and differences in CAA histopathological diagnosis.[153] A meta-analysis of these studies showed a statistically significant but imprecise association between CAA and lobar ICH (OR 2.2 95% CI 1.1 to 4.5). No association between deep ICH and CAA was identified (OR 0.8 95% CI 0.3 to 2.2).[153] CAA is therefore likely to account for only some lobar ICH, indicating that ICH location alone is insufficient for reliably identifying the underlying SVDs.

1.3.3 Genetics

As discussed in 1.1.1.2, APOE ε4 allele has a dose-dependent association with pathologically proven sporadic CAA.[24, 25] Possession of an APOE ε4 allele may, therefore, be useful for differentiating CAA-associated from arteriolosclerosis-associated ICH. However, the diagnostic value of APOE genotype on its own or in combination with other clinical and neuroimaging features for CAA-associated ICH is unknown.

1.3.4 Neuroimaging

Brain imaging is commonly performed in ICH. CT, MRI and positron emission tomography (PET) are neuroimaging techniques that can potentially help differentiate CAA-associated from arteriolosclerosis-associated ICH.

1.3.4.1 CT

CT is widely available, has few contraindications and is often the first test to diagnose ICH, yet no CT-based diagnostic criteria for CAA-associated or arteriolosclerosis-associated ICH currently exist.

A recent systematic review identified 23 studies (21 case series without controls and two cross-sectional studies with controls) that assessed CT

features in lobar or cerebellar ICH in 319 adults with pathologically proven CAA.[166] In the case series, subarachnoid haemorrhage was the most frequent CT feature in pathologically proven CAA (pooled proportion 82%, 95%CI 69-93%), followed by an irregular ICH border (64%, 95%CI 32-91%). Multiple acute ICHs were present in 37% (95%CI 18-58%). The frontal and parietal lobes were the most frequently affected by ICH, followed by the occipital lobe and then the temporal lobe. In one of the retrospective hospital-based cross-sectional studies of 41 cases and 42 controls, CAA-associated lobar ICHs were more likely to be multiple, have subarachnoid haemorrhage and a lobulated border. There was no association between subarachnoid haemorrhage and CAA-associated lobar ICH in the other cross-sectional study of 22 cases and nine controls.

Given its widespread availability, CT-based diagnostic criteria to differentiate CAA-associated from arteriolosclerosis-associated ICH would be very useful. CT features, such as subarachnoid haemorrhage and a lobulated ICH border may be useful for identifying CAA-associated lobar ICH. However, rigorous diagnostic test accuracy studies are required to assess this.

1.3.4.2 MRI

MRI is the *in vivo* reference standard for assessing SVDs.[77] A range of SVD biomarkers can be identified on MRI (1.1.3). The presence and distribution of these SVD biomarkers may be useful for identifying the types of SVDs underlying ICH.

The Boston criteria are MRI-based diagnostic criteria for CAA, which are frequently used in clinical practice and in research to guide further, often invasive, investigations, treatment decisions and recruitment into studies.[103, 110, 167, 168] These criteria use the number and distribution of haemorrhagic foci to determine the likelihood of underlying CAA.

The original Boston criteria were first published in 1995 and consider CMBs and macrohaemorrhages as haemorrhagic foci (Table 1.1).[169] Three external validation studies of the original Boston criteria in ICH participants showed good to excellent specificity (81%-100%) and moderate to good

sensitivity (73-90%) against a histopathological reference standard.[103, 110] However, all these studies were small with significant methodological limitations. In 2010, cortical superficial siderosis was added to the criteria as a further potential haemorrhagic focus to create the modified Boston criteria (Table 1.1), which are now the clinical reference standard for diagnosing CAA. While the modified Boston criteria showed excellent sensitivity and good specificity for CAA-associated ICH during their development,[103, 110] they have never been rigorously externally validated. Their true diagnostic accuracy is, therefore, unknown.

1.3.4.3 Positron emission tomography

CT and MRI can detect structural brain changes secondary to SVDs, such as CMBs and macrohaemorrhages, subarachnoid haemorrhage and cortical superficial siderosis, WMH and perivascular spaces. The presence and distribution of these imaging biomarkers may be useful to infer the underlying SVD type. However, the imaging biomarkers associated with arteriolosclerosis and CAA are likely to overlap given their overlapping brain distribution and shared pathophysiology, thus limiting their specificity. Also, these structural changes are likely to represent relatively late consequences of SVDs. In particular, haemorrhages are thought to be a late consequence of advanced CAA.[170] Therefore, structural imaging biomarkers may not be sensitive for identifying those patients in the earliest stages of these diseases.

Positron emission tomography (PET) is a form of *in vivo* molecular imaging that may improve the identification of SVDs during life through the direct detection of specific molecular changes. For example, β-amyloid PET radioligands designed for Alzheimer's disease may be able to detect the perivascular β-amyloid in CAA,[171, 172] and may have better sensitivity and specificity for CAA-associated ICH than structural imaging biomarkers. However, the diagnostic accuracy of β-amyloid PET in SVD-associated ICH is not known. Well-designed diagnostic test accuracy studies are required.

Table 1.1 Comparison of the original and modified Boston criteria for CAA-associated haemorrhage

	Original Boston criteria	Modified Boston criteria
Probable CAA	• Aged 55 years or over • Multiple macrohaemorrhages or CMBs restricted to lobar, cortical or cortical-subcortical regions (cerebellar haemorrhage allowed) • Absence of other cause of haemorrhage	• Aged 55 years or over • Multiple macrohaemorrhages or CMBs restricted to lobar, cortical or cortical-subcortical regions (cerebellar haemorrhage allowed), or • Single lobar, cortical or cortical-subcortical macrohaemorrhage or CMB and focal or disseminated cortical superficial siderosis • Absence of other cause of haemorrhage or cortical superficial siderosis
Possible CAA	• Aged 55 years or over • Single lobar, cortical or cortical-subcortical macrohaemorrhage or CMB • Absence of other cause of haemorrhage	• Aged 55 years or over • Single lobar, cortical or cortical-subcortical macrohaemorrhage or CMB, or • Focal or disseminated cortical superficial siderosis • Absence of other cause of haemorrhage or cortical superficial siderosis
No CAA	• Cases not meeting the criteria for probable or possible CAA	• Cases not meeting the criteria for probable or possible CAA

CAA = cerebral amyloid angiopathy. CMB = cerebral microbleed

1.4 Ideal design of diagnostic test accuracy studies

Rigorous diagnostic test accuracy studies of CT, MRI and β-amyloid PET imaging biomarkers in SVD-associated ICH are required to determine their diagnostic value.[173] The ideal diagnostic test accuracy study design includes:

- Prospective study design.
- Using a representative sample of cases and controls drawn from the same population to minimise selection bias.
- Performing the index test and reference standard at standardised time points after the index ICH.
- Performing the same index test and reference standard to avoid differential verification bias.[174]
- Using systematic approaches for analysing the index test (assessing CT, MRI or β-amyloid PET imaging biomarkers) and reference standard with pre-specified definitions for imaging biomarkers and reference standard cut-offs to minimise information bias.
- Assessment of the index test and reference standard by several raters to determine inter-rater agreement.
- Masking of the raters to other relevant data.
- Pre-specifying the statistical analyses.
- Appropriately powered with sufficient numbers of participants with each target pathology to allow statistical analysis of the diagnostic test performance characteristics.
- Describing the flow of participants through the studies and the differences between those who did and did not undergo the reference standard to illustrate partial verification bias.[174, 175]
- Reporting the baseline clinical and radiographic features and the distribution of CAA and non-CAA SVD severity in the study groups to describe the spectrum of participants.[175, 176]

1.5 Aims

There are no effective acute or specific treatments for ICH. Better prevention of ICH (first-ever and recurrent) is likely to be a more promising strategy to decrease its burden. Identifying the types of SVDs underlying SVD-associated ICH is a fundamental step to increase our understanding of the disease processes. It may allow us to better recognise those at highest risk of death, disability and subsequent ICH, and help guide some treatment decisions.

The main aims of my thesis were to:

- Assess the incidence and distribution of clinical and non-contrast brain CT features in first-ever SVD-associated ICH according to ICH location in a community-based cohort of ICH (Chapter 3).
- Assess APOE genotype and the distribution of MRI SVD biomarkers in first-ever SVD-associated ICH according to ICH location in a research cohort study of ICH (Chapter 3).
- Evaluate the prevalence, severity and associations of histopathologically assessed SVDs in first-ever SVD-associated lobar ICH according to ICH location in a research cohort study of ICH (Chapter 4).
- Assess the diagnostic accuracy of the MRI-based Boston criteria for CAA-associated ICH against a histopathological reference standard in a research cohort study of ICH (Chapter 5).
- Investigate the feasibility and utility of β-amyloid MRI-PET in first-ever SVD-associated ICH (Chapter 6).
- Assess the diagnostic accuracy of CT biomarkers and APOE genotype for diagnosing first-ever CAA-associated lobar ICH and derive multivariable diagnostic models and criteria (The Edinburgh Diagnostic Criteria) for first-ever CAA-associated lobar ICH (Chapter 7).
- Perform multicentre external validation studies of the Edinburgh diagnostic models for CAA-associated with lobar ICH, and assess the

diagnostic accuracy of the Edinburgh Diagnostic Criteria against a histopathological reference standard (Chapter 8).

- Assess the diagnostic accuracy of the Edinburgh Diagnostic Criteria for CAA-associated lobar ICH against the modified Boston criteria (Chapter 9).
- Investigate the association between the Edinburgh Diagnostic Criteria for CAA-associated lobar ICH and the risk of recurrent ICH in survivors of lobar ICH (Chapter 10).
- Develop multivariable logistic regression prognostic models to predict the risk of death and death or disability after first-ever SVD-associated ICH, and compare these against the ICH score (Chapter 11).

Section B. Methods

Chapter 2 Methods

Chapter 2 Methods

To investigate the diagnostic and prognostic value of SVD imaging biomarkers in SVD-associated ICH, I used data from two overlapping cohort studies; The Lothian Audit of the Treatment of Cerebral Haemorrhage (LATCH) and The Lothian INtraCerebral Haemorrhage Pathology, Imaging and Neurological outcome (LINCHPIN) study. The studies were conducted by the Research to Understand Stroke due to Haemorrhage (RUSH) team at the University of Edinburgh. This chapter describes the design of these studies, including methods of case ascertainment, baseline data collection and follow-up. I will also describe the methods used for brain CT and MRI acquisition and analysis, DNA extraction and APOE genotyping and research brain autopsy.

2.1 Cohort studies

2.1.1 Overview of studies

LATCH is a community-based cohort study of all residents in the NHS Lothian Health Board region of Scotland who were aged 16 years or above and had an incident ICH between 1st June 2010 and 31st May 2013 inclusive.

The purpose of LATCH was to monitor the quality of care, treatment and outcomes for adults with ICH. Its primary aim was to audit neurosurgical referrals and prescription of antihypertensive drugs at hospital discharge against European[177] and NICE guidelines.[178] Observational epidemiology can be performed using an anonymised extract of the audit dataset to examine the clinical and imaging characteristics of ICH as well as the factors that are associated with prognosis after ICH in a community-based study. LATCH aimed to identify all ICH regardless of cause, including those due to a macrovascular or neoplastic cause as well as those without any detectable macroscopic cause (SVD-associated ICH) (Table 2.1).

During LATCH and until 31st May 2016, consecutive adults with SVD-associated ICH were given the opportunity to consent to the LINCHPIN study (Figure 2.1). LINCHPIN is an ethically-approved, prospective, community-based cohort study of SVD-associated ICH designed to pathologically assess the SVDs associated with SVD-associated ICH, determine the diagnostic utility of genetic and SVD biomarkers on brain CT and MRI for diagnosing pathologically-proven CAA, and assess the prognostic value of genetic and imaging SVD biomarkers in ICH. Eligible participants were invited to consent to any of the components of LINCHPIN (Figure 2.2):

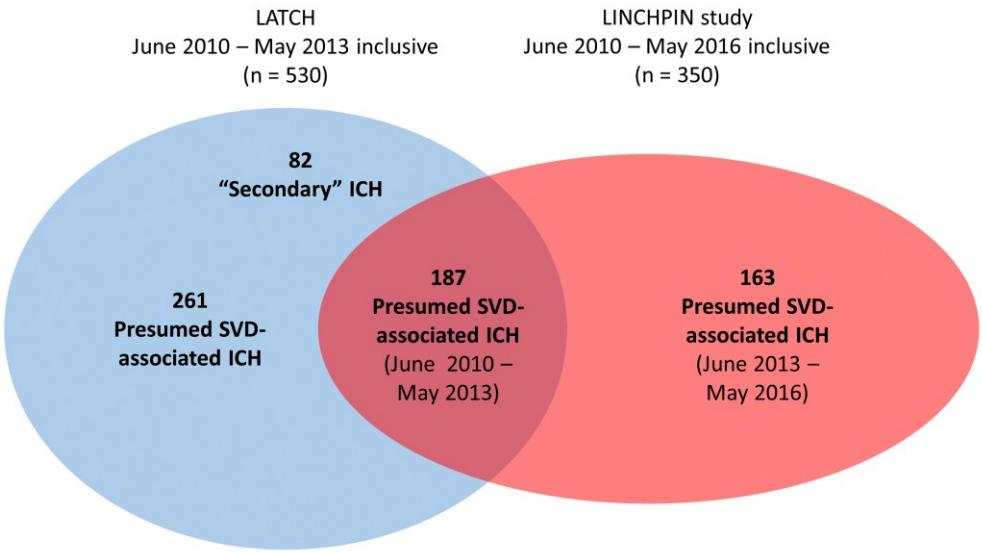
- A clinical assessment.
- Permission for the research team to access their medical records.
- Apolipoprotein E genotyping.
- Research brain MRI scan.
- Research autopsy limited to the brain.

Table 2.1 Structural causes of intracerebral haemorrhage in LATCH.

Cause of intracerebral haemorrhage		**Frequency**	**(%)**
SVD-associated ICH	No neoplastic or macrovascular cause	448	84.5
“Secondary” ICH	Malignancy	31	5.8
	Arterial aneurysm	21	4.0
	Arteriovenous malformation	16	3.0
	Cavernous malformation	8	1.5
	Intracranial venous thrombosis	3	0.6
	Dural arteriovenous fistula	2	0.4
	Abscess	1	0.2

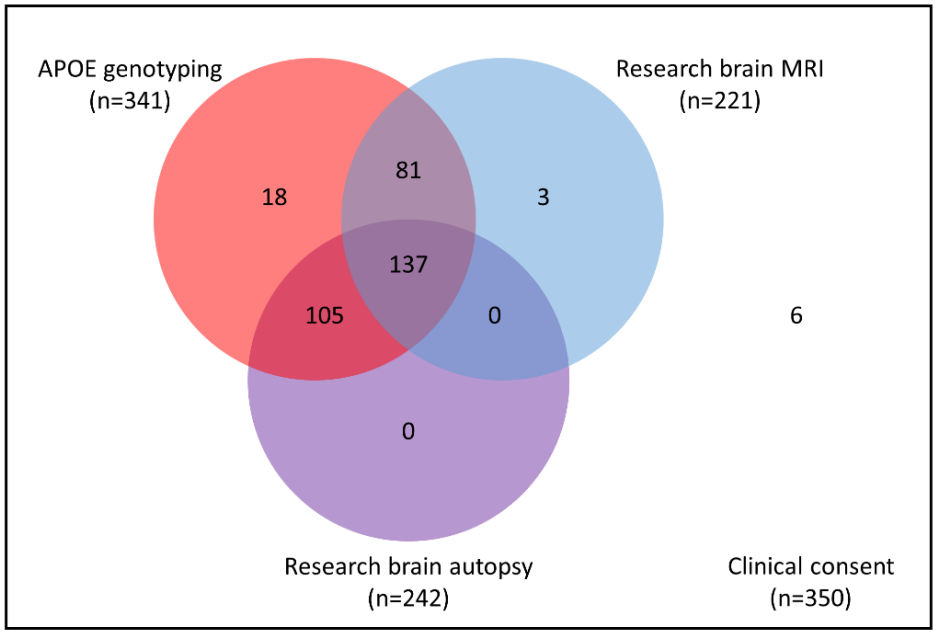
ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. SVD = small vessel disease.

Figure 2.1 The overlap between LATCH and the LINCHPIN study



ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. SVD = small vessel disease.

Figure 2.2 The numbers of participants consenting to the different components of the LINCHPIN study



APOE = apolipoprotein E. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. MRI = magnetic resonance imaging.

2.1.2 Study settings

Both LATCH and LINCHPIN were set in the NHS Lothian Health Board region of Scotland. According to Scotland's Census 2011, the total population within this region was 834,350, with 51.4% females. 692,542 were aged 16 or above.[179]

NHS Lothian has three hospitals with emergency departments or acute medical receiving units (Royal Infirmary of Edinburgh, Western General Hospital, Edinburgh and St John's Hospital, Livingston). All three hospitals have in-patient and out-patient stroke services, including acute stroke units.

2.1.3 Study inclusion and exclusion criteria

2.1.3.1 LATCH

Patients were included in LATCH if they met all of the following:

- Incident first-ever or recurrent spontaneous ICH.
- ICH was confirmed by brain imaging or autopsy.
- ICH symptom onset between 1st June 2010 and 31st May 2013 inclusive.
- Aged 16 years or over at ICH onset.
- Resident within NHS Lothian Health Board region.

Patients with any of the following were excluded from LATCH:

- ICH was definitely secondary to trauma.
- ICH was definitely secondary to haemorrhagic transformation of an ischaemic stroke (HTI).
- Exclusively extra-axial intracranial haemorrhage (i.e. extradural, subdural, subarachnoid or intraventricular haemorrhage).

2.1.3.2 LINCHPIN

Patients were invited to consent to the LINCHPIN study if they met all of the following:

- Incident first-ever or recurrent spontaneous ICH.
- ICH was confirmed by brain imaging or autopsy.

- No evidence of underlying cause other than SVDs.
- ICH symptom onset between 1st June 2010 until and 31st May 2016 inclusive.
- Aged 16 years or over at ICH onset.
- Resident within NHS Lothian Health Board region.

Patients with any of the following were excluded from LINCHPIN:

- ICH was definitely secondary to trauma.
- ICH was definitely secondary to a neoplastic, abscess or macrovascular cause.
- ICH was definitely secondary to HTI.
- Exclusively extra-axial intracranial haemorrhage (i.e. extradural, subdural, subarachnoid or intraventricular haemorrhage).

2.1.4 Sources of ascertainment

The RUSH team used multiple overlapping sources of case ascertainment to identify all incident ICH cases during the study periods.

Prospective methods involved:

- A collaborative network of acute and emergency medicine physicians, neurologists, stroke physicians and stroke nurse specialists, neurosurgeons, radiologists, rehabilitation physicians and pathologists who prospectively identified adults with incident ICH who attended hospital or neurovascular clinic.
- Weekday review of all brain CT scans and their reports in the NHS Lothian Health Board region by trained members of the RUSH team to identify adults with ICH.

Retrospective methods used by the RUSH team involved:

- Six monthly review of the Scottish Stroke Care Audit, a national audit of stroke management and outcome in Scotland.[180]
- Annual review of ICD-10-coded records of hospital discharges held by the Information Services Division.

- Annual review of records of sudden deaths held by the Office of the Procurator Fiscal.

2.1.5 Diagnosis of ICH

ICH was defined as the abrupt symptomatic onset of severe headache, altered level of consciousness, or focal neurological deficit, anatomically referable to a focal collection of blood within the brain parenchyma as observed on brain imaging (CT or MRI) or at autopsy, with characteristics consistent with the time of symptom onset, which was not attributable to prior trauma or HTI.[40]

2.1.5.1 Traumatic ICH vs spontaneous ICH

Patients with ICH can present with a history of trauma. Determining whether the ICH is the cause or a consequence of trauma can be difficult. Several approaches were used to differentiate spontaneous ICH from traumatic ICH:

- Clinical history – A history of stroke symptoms before trauma suggests a spontaneous ICH, while a history of major trauma without preceding stroke symptoms suggests a traumatic ICH.
- Physical examination – Signs of significant head trauma, such as clear fluid (CSF) running from the ears or nose, bleeding from the ears, bruising around the orbits or ears and scalp haematoma, suggest a traumatic ICH.
- Radiographic – Features on non-contrast CT imaging suggestive of trauma include haemorrhagic cerebral contusions. These are hyperattenuating areas within the parenchyma which represent acute haemorrhage. They are typically located in the inferior frontal lobes and temporal pole, areas which are adjacent to the skull.[181, 182] A coup-contrecoup pattern of subarachnoid haemorrhage and parenchymal contusions may be present, where there is haemorrhage at both the site of impact and the opposite side.

Spontaneous ICH can result in a fall and traumatic brain injury. Therefore, patients may have features of both spontaneous and traumatic ICH. These types of cases were included as the initial haemorrhage was spontaneous.

2.1.5.2 HTI vs spontaneous ICH

Ischaemic stroke can subsequently undergo petechial or parenchymal haemorrhagic transformation and be confused with spontaneous ICH. Differentiating HTI from spontaneous ICH was straightforward when there was baseline imaging showing an acute or subacute ischaemic stroke and subsequent imaging showing haemorrhage within the infarcted tissue. It was more difficult when the patient presented subacutely, and haemorrhage was present on the baseline imaging. In this situation, HTI was suspected when petechial or parenchymal haemorrhage occurred in a region with features of a subacute infarct on brain imaging, such as a hyperattenuating artery and/or a region of low attenuation which involved both cortex and white matter, with positive mass effect and was confined to an expected arterial territory.

2.1.5.3 Stroke imaging meeting review

A combination of clinical and imaging features was used to determine whether an ICH was HTI, traumatic, spontaneous or a combination of spontaneous and traumatic. Neurologists, stroke physicians and neuroradiologists discussed the cases in a weekly multidisciplinary stroke imaging meeting, and a consensus decision was reached based on all available evidence. Cases where further investigations were recommended were re-discussed at the stroke imaging meeting once all investigations were complete.

2.1.6 ICH onset date

The ICH onset date was defined as the date when symptoms attributable to the ICH began or when the patient was last seen well, if symptom onset was unknown. It was determined by the RUSH team through interviewing the patient or their relatives and reviewing hospital records. The ICH onset date was not necessarily the same as the date of hospital admission or diagnostic scanning. The ICH onset date was the inception point for the study and the point from which prospective follow-up began.

In rare cases where ICH was identified incidentally, the ICH onset date was the date of the hospital admission or outpatient clinic which resulted in the imaging.

2.1.7 Baseline data collection

The RUSH team recorded demographics, comorbidities, baseline clinical data and medication use at the time of index ICH (Table 2.2) by interviewing patients and their families at the time of presentation, and through review of primary and secondary care records. Where possible, multiple sources were used to corroborate information.

2.1.7.1 Co-morbidities

The condition and the date of diagnosis were recorded. When the date of diagnosis was not known, the following approaches were used

- If the day of diagnosis was unknown but the month and year were known, then the midpoint of the month was entered (i.e. 15/mm/yyyy).
- If the day and month were unknown but the year was known then the midpoint of the year was entered (i.e. 01/07/yyyy).
- If the day, month and year were unknown, then no date was entered.

Table 2.2 Completeness of selected baseline clinical characteristics collected in LATCH and the LINCHPIN study.

Clinical characteristics	Response (%)	
	LATCH SVD-associated ICH (n=448)	LINCHPIN SVD-associated ICH (n=351)
Age (years)	100.0	100.0
Sex	100.0	100.0
Co-morbidities		
Hypertension	99.8	100.0
Hyperlipidaemia	99.8	100.0
Atrial fibrillation	99.8	100.0
Myocardial infarction	99.8	100.0
Ischaemic stroke	99.8	100.0
Transient Ischaemic Attack	99.8	100.0
Dementia	100.0	100.0
Diabetes	99.8	100.0
Smoking status	99.1	100.0
Alcohol intake	94.6	84.9
Pre-ICH modified Rankin scale	98.9	99.4
Medications on admission		
Antiplatelet drug(s)	100.0	100.0
Anticoagulant drug(s)	100.0	100.0
Antihypertensive drug(s)	100.0	100.0
Admission Glasgow coma scale	99.1	99.4
Admission systolic blood pressure	94.6	95.4
Admission diastolic blood pressure	94.2	97.2

ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. SVD = small vessel disease.

A history of hypertension was defined as either a documented diagnosis of hypertension in the medical records or if the patient was taking antihypertensive medications at the time of their ICH.

A patient was classified as having a history of hyperlipidaemia when there was a documented diagnosis of hyperlipidaemia in their medical records. The

use of a statin medication at the time of their ICH on its own was not sufficient for a history of hyperlipidaemia, as these medications are also used in patients without hyperlipidaemia.

A history of dementia was defined as either a diagnosis of dementia in their medical records or if a relative or close friend completed the short form Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) and the score was ≥64.[183]

A history of diabetes mellitus was defined as either a diagnosis of diabetes mellitus, or prescription of a sulphonylurea or insulin in their medical records, or evidence in the medical records that the World Health Organization 2011 criteria for diabetes mellitus had been fulfilled.[184]

Patients with a history of diabetes mellitus were further classified as:

- Type 1 diabetes. If this was not specified, it was assumed if the diagnosis was made before the age of 30 years and treated with insulin therapy alone.
- Type 2 diabetes. If this was not specified, it was assumed if the patient was on diet control or oral hypoglycaemic drugs alone, or diabetes diagnosed after the age of 30 years.
- Diabetes, type unknown was recorded if there was insufficient information to determine the type.

2.1.7.2 Pre-ICH level of functioning

The pre-ICH level of functioning was assessed by a trained rater (Dr N Samarasekera, C Lerpinere or Professor R Al-Shahi Salman) by interviewing patients and their families at the time of presentation and was quantified using the modified Rankin scale[185].

2.1.7.3 Clinical data on admission

The first systolic and diastolic blood pressure and Glasgow Coma Scale (GCS) score on hospital admission were recorded from the medical records or clinic assessment (Table 2.2).

2.1.7.4 Investigations

Results from the first blood samples (full blood count, renal profile, coagulation screen) taken on the day of admission or clinic assessment were recorded.

The date, time and type of all diagnostic brain imaging, such as non-contrast CT, CT angiography, CT venography and MRI, were recorded. The underlying structural cause of the ICH after diagnostic workup was also documented.

2.1.8 Follow-up data collection

2.1.8.1 Ascertainment of follow-up data

The RUSH team used multiple sources of information to follow-up participants in LATCH and LINCHPIN.

- Annual GP postal questionnaire: This asked the GP about the occurrence of any major vascular outcomes listed below, development of dementia or hypertension and the level of functioning, based on the modified Rankin scale. The GP was also asked to send the most recent GP electronic summary of co-morbidities and current prescriptions, as well as the most recent blood pressure recording.
- NHS Lothian's secondary care electronic records system (TRAK): This was reviewed annually by members of the RUSH group for vital status and occurrence of the relevant outcomes listed below.
- Scottish National Picture Archive and Communication System (PACS): This was reviewed annually for the occurrence of the relevant vascular outcomes listed below.
- GP primary care records: The RUSH team requested and reviewed these in all participants who died.

Follow-up is still ongoing for all surviving LATCH and LINCHPIN participants.

2.1.8.2 Outcomes of interest

The outcomes recorded were death, functional outcome according to the modified Rankin scale,[185] major vascular outcomes, such as ischaemic

stroke, transient ischaemic attack, deep vein thrombosis/pulmonary embolism, acute coronary syndrome, ICH or other intracranial and extracranial bleeding, and development of dementia.

2.1.8.3 Outcome adjudication

All outcomes for death and major vascular events were adjudicated by Professor R Al-Shahi Salman, a consultant neurologist with an interest in stroke medicine.

- For deaths, the death certificate, GP and hospital records, and autopsy report, if performed, were reviewed to confirm the date and cause of death.
- For major vascular outcomes, any GP or hospital records and any relevant imaging on the Scottish National PACS relating to the outcome were reviewed to confirm the type and date of the outcome.

2.1.9 Regulatory approvals

The NHS Lothian Caldicott Guardian approved LATCH. Patients in NHS Lothian were informed about the use of their data for audit, and information leaflets about LATCH were distributed to inform patients and their carers about their right to opt out.[40] Analysis of an anonymised data set did not require research ethics committee approval.

LINCHPIN was approved by the Scotland A Research Ethics Committee (10/MRE00/23). The RUSH team obtained written informed consent from all participants or their nearest relative or welfare guardian when participants did not have mental capacity.

2.2 Image acquisition and analysis

2.2.1 Diagnostic non-contrast brain CT scan

2.2.1.1 Scan acquisition

The diagnostic non-contrast brain CT scans in LATCH and LINCHPIN were performed in NHS Lothian as part of routine clinical practice. CT scan

parameters (tube current and voltage, default windowing levels) varied between the radiology departments at the three hospitals within NHS Lothian. All diagnostic non-contrast brain CT scans were acquired as volumetric scans, allowing reconstruction in any plane.

2.2.1.2 Scan reformatting

It was not always possible to use standard patient positioning in the CT scanner, particularly for acutely unwell patients who for example, may only be able to lie on their side rather than on their back. I therefore, reformatted the first diagnostic non-contrast brain CT scan performed after ICH onset using standard planes for consistency (Figure 2.3 and Figure 2.4):

- Axial: Parallel to a line linking the floor of the sella turcica to the fastigium of the fourth ventricle in the sagittal plane and parallel to the inferior aspect of the temporal poles in the coronal plane.
- Coronal: parallel to the posterior surface of the brainstem in the sagittal plane and perpendicular to the interhemispheric fissure in the axial plane.
- Sagittal: parallel to the interhemispheric fissure in both the coronal and axial planes.

I standardised the slice thickness (5mm), spacing (3mm) and windowing (centre 35, width 80). I chose the slice thickness and spacing to make the CT images comparable to the 2D MRI sequences acquired as part of the research MRI scans. The slice thickness also increases signal to noise ratio.

I performed the reformatting of the diagnostic non-contrast brain CT scans using Carestream Vue PACS version 11.3.2, Carestream Health, Inc, USA.

2.2.1.3 Image analysis

I rated the reformatted first diagnostic non-contrast brain CT scan performed after ICH onset using a standardised pro forma (Appendix 1) modified from previous large-scale stroke studies[186-189] to include the assessment of recommended CT imaging features of SVDs[77] and CT imaging features

associated with pathologically proven CAA.[166] If necessary, I also reviewed the source volumetric CT data if required to complete the ratings.

ICH features

I recorded the location of all acute ICHs using the Cerebral Haemorrhage Anatomical RaTing Scale (CHARTS),[190] a published tool for determining ICH location. I assessed the volume of each ICH using the ABC/2 method, where A is the largest ICH diameter in the axial plane in centimetres, B is the largest diameter perpendicular to A on the same slice in centimetres, and C is the maximal cranio-caudal diameter in centimetres.[191] I recorded the presence or absence of haemorrhage within the ventricular, subarachnoid or subdural spaces. I assessed the presence or absence of three separate features of ICH shape; finger-like projections, defined as elongated extensions arising from the haematoma that are longer than they are wide, an irregular contour, and a round/oval shape (Supplementary Appendix in Chapter 7 and Appendix 1).[166]

I recorded whether there was a fluid level within the haematomas and whether they had a dilute or “seeping” appearance, as well as whether the ICH reached the cortex.[166, 192] I defined dilute/“seeping” as adjacent areas of relative hypoattenuation and hyperattenuation within a haematoma with a well-defined margin between these regions. I used representative images to define the features associated with ICH shape and appearance, such as irregular contour, finger-like projection and dilute/”seeping” (Appendix 1).

Brain SVDs features

I recorded the presence, type and location of old infarcts, the severity of anterior and posterior white matter lucencies using the van Swieten scale,[193] and deep (enlargement of the ventricles) and superficial (enlargement of the sulci) cerebral atrophy using a template based three-point scale (0 = absent/mild, 1 = moderate and 2 = severe).[186, 194, 195]

I calculated a CT SVD burden score based on the presence and severity of white matter lucencies, lacunes and atrophy.[196] 1 point was awarded for each of the following if present:

- Severe lucencies (van Swieten Scale = 2) in anterior or posterior periventricular white matter
- ≥2 lacunes
- Severe (=2) central or cortical atrophy

The combined 4-point ordinal score, therefore, assessed the global burden of SVD from 0 (no imaging features of severe SVDs) to 3 (imaging features of SVDs scored as severe for each imaging variable). The biological plausibility and prognostic value of SVDs burden scores have been demonstrated on MRI.[186, 197, 198]

CT rating

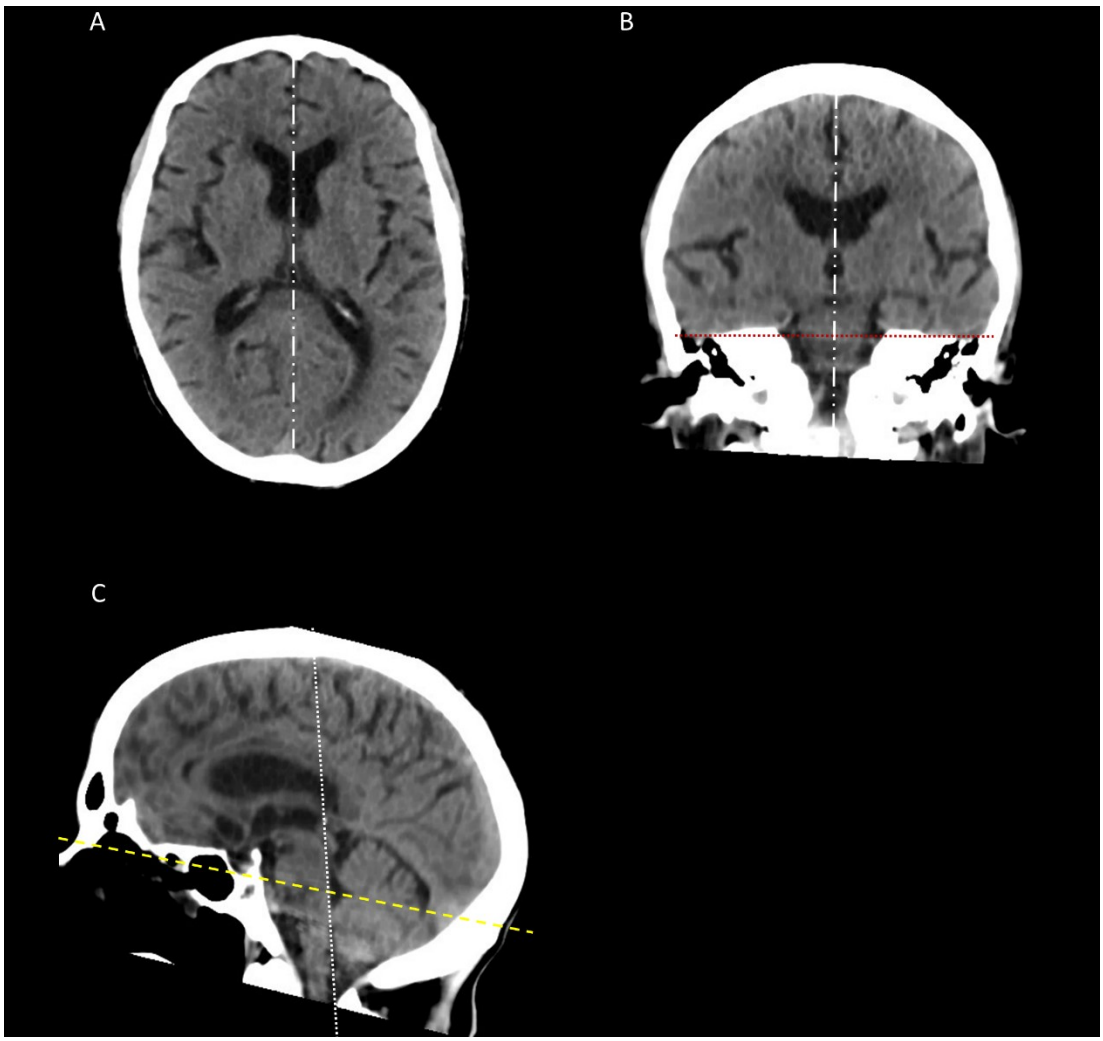
I performed the visual ratings of the diagnostic non-contrast brain CT scans using Carestream Vue PACS version 11.3.2, Carestream Health, Inc, USA. I performed all assessments masked to clinical, genetic, MRI, histopathological and outcome data.

Figure 2.3 Standard planes used to reformat the first diagnostic non-contrast brain CT scan

A. Axial plane. The white dot-dashed line indicates the plane parallel to the interhemispheric fissure used to align the coronal and sagittal planes.

B. Coronal plane. The white dot-dashed line indicates the plane parallel to the interhemispheric fissure used to align the sagittal plane. The red dotted line indicates the plane parallel to the inferior aspect of the temporal poles used to align the axial plane.

C. Sagittal plane. The white dotted line indicates the plane parallel to the posterior surface of the brainstem used to align the coronal plane. The yellow dashed line indicates the plane parallel to a line linking the floor of the sella turcica to the fastigium of the fourth ventricle used to align the axial plane.



CT = computed tomography.

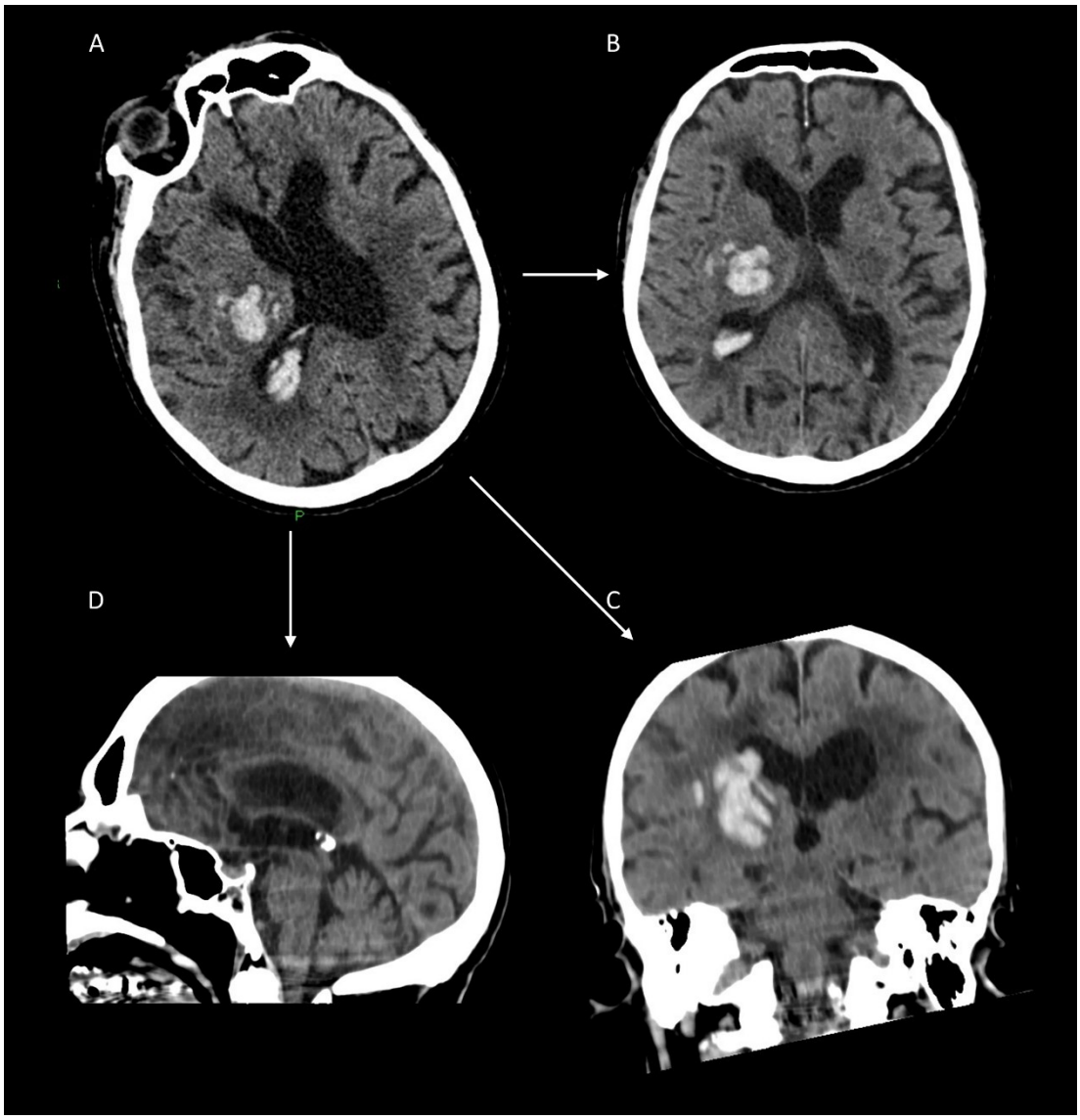
Figure 2.4 Reformatting of first diagnostic non-contrast brain CT scan

A. Unformatted diagnostic non-contrast brain CT.

B. Reformatted axial plane

C. Reformatted coronal plane

D. Reformatted sagittal plane



CT = computed tomography.

2.2.2 Diagnostic (clinical) brain MRI scan

In a small number of patients, the first diagnostic scan performed after ICH was a brain MRI, typically because of a delayed presentation or mild or atypical symptoms. The diagnostic brain MRI scans were performed in NHS Lothian as part of routine clinical practice. MRI scan parameters varied between the radiology departments within the three hospitals in NHS Lothian, although all scans were performed on 1.5T scanners.

For these patients, I assessed the number, location[190] and volume[191] of acute ICHs as described above. I also recorded the presence of haemorrhage within the ventricular, subarachnoid or subdural spaces.

2.2.3 Research LINCHPIN brain MRI scan

2.2.3.1 Eligibility

All participants within the LINCHPIN study were given the opportunity to consent to a research brain MRI. LINCHPIN participants were excluded from research brain MRI if they had any of the following:

- A contraindication to MRI, such as a non-MRI compatible implantable device or metallic foreign bodies in the eye.
- They were unable to tolerate MRI scanning because they were claustrophobic, unable to lie flat or were too unwell.
- They were unable to fit into the scanner.
- They had died before ascertainment.

2.2.3.2 Scan acquisition

LINCHPIN research brain MRIs were performed around six months from the index ICH on one of two 1.5T MRI scanners (GE Signal LX EchoSpeed scanner, Milwaukee, WI, USA at the Brain Research Imaging Centre, Western General Hospital, Edinburgh, and Philips Gyroscan Intera scanner, Philips Ltd, Best, The Netherlands at the Royal Infirmary of Edinburgh). The MRI protocol comprised of sagittal T1-weighted, and axial T2-weighted, FLAIR and T2*-weighted gradient recalled echo (GRE) sequences for all participants. Axial susceptibility weighted imaging (SWI), susceptibility

weighted angiography (SWAN) and diffusion-weighted imaging (DWI) sequences were later added to the MRI protocol. Therefore, some participants also had these sequences as well as the basic protocol described above. Standardised sequence parameters were used for all participants (Table 2.3 and Table 2.4).

2.2.3.3 Image analysis

I rated LINCHPIN brain MRI scans using a standardised pro forma (Appendix 2). I defined neuroimaging features of SVDs (lacunes, WMH, perivascular spaces, CMB, cortical superficial siderosis and brain atrophy) according to STRIVE (section 1.1.3).[77]

Acute ICH

I defined the age of an ICH according to the recognised MRI characteristics of the breakdown of haemoglobin with time.[199] I recorded the location and volume of all acute ICHs using CHARTS[190] and the ABC/2 method,[191] respectively, described in Section 2.2.1.3.

Acute ischaemic infarct

I recorded the number and location of acute infarcts, defined as regions of DWI hyperintensity with hypointensity on the corresponding apparent diffusion coefficient map, and normal or hyperintense signal on T2-weighted and FLAIR sequences.[186-188, 200-202]

Chronic infarcts and haemorrhages

I recorded the number and location of chronic infarcts and haemorrhages. I defined chronic infarcts as lesions with T2-weighted and FLAIR hyperintensity and T1-weighted hypointensity in an expected arterial territory. I defined lacunes as a round or ovoid, subcortical lesions between 3 and 15 mm in diameter in the territory of one perforating arteriole.[77] I defined chronic haemorrhages as slit-like cavities or areas of gliosis with peripheral hypointensity on blood-sensitive sequences, such as T2*-weighted GRE.[203]

Table 2.3 LINCHPIN study research brain MRI sequence parameters at the Brain Research Imaging Centre, Western General Hospital, Edinburgh

Sequence	**T1 SE**	**T2*GRE**	**T2 FSE with propeller**	**FLAIR**	**SWAN**	**SWI**	**DWI**
Orientation	SAG	AC-PC	AC-PC	AC-PC	AC-PC	AC-PC	AC-PC
TE	MIN	40	86.2	140	50	25	MIN
TR	380	300	5000	9000	78.9	5000	10000
TI				2000			
Flip angle		20			15	90	
Field of view	24	24	24	24	24	24	24
Slice thickness (mm)	5	5	5	5	3.8	3	5
Slice gap (mm)	1.5	1.5	1	1	0	0	1
Matrix	384x256	384x224	384x384	384x224	288x224	224x352	128x128

AC-PC = anterior commissure-posterior commissure. DWI = diffusion-weighted imaging. FLAIR = fluid-attenuated inversion recovery. FSE = fast spin echo. GRE = gradient recalled echo. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. MIN = minimum. MRI = magnetic resonance imaging. SAG = sagittal. SWAN = susceptibility weighted angiography. SWI = susceptibility weighted imaging. TE = echo time. TI = inversion time. TR = repetition time.

Table 2.4 LINCHPIN study research brain MRI sequence parameters at the Royal Infirmary of Edinburgh

Sequence	**T1 SE**	**T2*GRE**	**T2 FSE with propeller**	**FLAIR**
Orientation	SAG	AC-PC	AC-PC	AC-PC
TE	15	40	100	100
TR	623.0	300	4841.6	6000
TI				2000
Flip angle		20		
Field of view	23	24	23	23
Slice thickness (mm)	5	5	5	5
Slice gap (mm)	1	1.5	1	1
Matrix	256x205	268x166	384x242	256x161

AC-PC = anterior commissure-posterior commissure. DWI = diffusion-weighted imaging. FLAIR = fluid-attenuated inversion recovery. FSE = fast spin echo. GRE = gradient recalled echo. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. MIN = minimum. SAG = sagittal. MRI = magnetic resonance imaging. SWAN = susceptibility weighted angiography. SWI = susceptibility weighted imaging. TE = echo time. TI = inversion time. TR = repetition time.

WMH

I rated WMH using the Fazekas scale.[89] The Fazekas scale differentiates periventricular and subcortical WMH, which is potentially relevant for characterising the types of SVDs associated with ICH. Also, it has been widely used in previous stroke trials.

Enlarged perivascular spaces

I assessed enlarged perivascular spaces using a validated qualitative rating scale.[204] I used a scale of zero to four to rate the number of enlarged perivascular spaces in four different brain regions - the hippocampus, basal ganglia, centrum semiovale and midbrain - where zero = no enlarged

perivascular spaces, one = ≤10, two = 11-20, three = 21-40 and four = >40. For each region, I rated the number of perivascular spaces on each side before using the mean to produce an overall score for each region.

CMB

I assessed CMBs by modifying two validated qualitative rating scales. The Brain Observer MicroBleed Scale (BOMBS) was developed on T2*-weighted GRE MRI sequences at 1.5T from 264 adults with stroke or transient ischaemic attack and tested on a separate cohort of 156 adult stroke patients.[205] BOMBS rates the total number of CMBs in the following areas: supratentorial cortex/grey-white matter junction, supratentorial subcortical white matter, basal ganglia, internal and external capsule, thalamus, brainstem and cerebellum. It distinguishes definite and possible CMBs as well as those <5 mm in diameter versus 5-10 mm. The Microbleed Anatomical Rating Scale (MARS) was developed on 301 consecutive stroke patients with T2*-weighted GRE MRI sequences at 1.5T.[206] Like BOMBS, MARS distinguishes definite from possible CMBs in the basal ganglia, thalamus, brainstem and cerebellum. MARS differentiates the different cerebral lobes (frontal, parietal, temporal, occipital and insula), but does not distinguish cortical and subcortical lobar CMBs. It also has separate categories for the corpus callosum and deep and periventricular white matter and includes a total number of CMBs in lobar, deep and infratentorial regions.

I added individual cerebral lobe categories (frontal, parietal, temporal and occipital lobes) to BOMBS, as this may be informative given the perceived posterior predominance of CMBs in CAA.[27, 207, 208] Also, I included totals for lobar, deep and infratentorial locations, which may be relevant to differentiate different types of SVDs given the typical distribution of CAA and non-CAA SVDs.[1, 153] I included deep and periventricular white matter (defined as adjacent to or within approximately 10 mm of the lateral ventricular margins) in the deep region given that small penetrating arteries supply all these regions. I only recorded definite CMBs given this improved inter-rater agreement in both BOMBS and MARS.[205, 206] I also recorded

the number of CMBs visible on SWI given its higher sensitivity and reliability for CMBs compared with T2*-weighted GRE.[209] I did not record CMB size given the current definition of CMBs in consensus guidelines for neuroimaging features of SVDs.[77] I considered potential CMB mimics when rating CMBs, such as flow voids in small cortical vessels, mineralization in the globi pallidi or dentate nuclei and partial volume effects adjacent to the petrous apex and orbit, particularly when I identified only one CMB.[205, 206]

Cortical superficial siderosis

I rated the location, extent (number of sulci involved) and proximity to index ICH (adjacent, distant, mixed) of cortical superficial siderosis using T2*-weighted GRE, and SWI when available.

Brain atrophy

I recorded deep (enlargement of the ventricles) and superficial (enlargement of the sulci) cerebral atrophy using a validated template-based approach (based on T2-weighted images of 105 subjects aged 75-80).[194] I used a scale of one to six, where one = ≤5th centile on atrophy template, two = 25th – 50th centile, three = 50th – 75th centile, four = 75th – 95th centile, five = >95th centile and six = >>95th centile.

Boston criteria for CAA

I used the original and modified Boston criteria to classify each brain MRI scan as showing probable CAA, possible CAA or no CAA based on the presence and distribution of macrohaemorrhages, CMBs and cortical superficial siderosis as described in Table 1.1.[110]

There is ambiguity over the implementation of the original and modified Boston criteria. Firstly, it is not clear whether cerebellar haemorrhages should be considered as a compatible haemorrhagic focus for probable CAA or possible CAA. The published original and modified Boston criteria specifically state that cerebellar haemorrhage is permitted in the multiple haemorrhage criterion of probable CAA. However, there is no mention of cerebellar haemorrhage, either positively or negatively, in the rest of the criteria. In my thesis I considered macrohaemorrhages and CMBs within the cerebellum as

compatible haemorrhagic foci for both probable CAA and possible CAA when using the original and modified Boston criteria.

Secondly, the modified Boston criteria include focal or disseminated cortical superficial siderosis as a compatible haemorrhagic focus for probable CAA or possible CAA, without specifying any restriction over its proximity to macrohaemorrhages or CMBs. Therefore, I considered any focus of cortical superficial siderosis as an independent haemorrhagic focus when applying the modified Boston criteria, regardless of its relationship with other haemorrhagic foci.

MRI brain SVD burden score

The MRI brain SVD burden score is an ordinal score of four MRI SVD biomarkers: lacunes, WMH, CMBs and enlarged perivascular spaces. It provides a "total SVD burden" score. It was developed in a prospective cohort of 122 participants with first-ever lacunar stroke who underwent standardised 1.5T or 3T MRI.[210] The scans were assessed independently by two vascular neurologists for asymptomatic lacunes, CMBs, enlarged perivascular spaces and WMH. One point is scored for each of the following if present:

- ≥1 lacune
- ≥1 CMBs
- Moderate to severe perivascular spaces in the basal ganglia (grade 3 or 4 – see Section 2.2.3.3)
- Periventricular WMH Fazekas 3 and/or deep WMG Fazekas 2-3 (see Section 2.2.3.3)

The combined 5-point ordinal score, therefore, assesses the global burden of SVDs from a minimum score of 0 to a maximum score of 4.

The validity of the MRI brain SVD burden score was initially assessed in 222 participants with acute lacunar stroke and 239 with mild cortical ischaemic stroke.[197] The MRI brain SVD burden score is associated with an increased risk of recurrent ischaemic stroke and ICH in patients with previous

TIA or ischaemic stroke,[211] mortality after acute ischaemic stroke[212] and predicts cognitive impairment.[198, 213]

MRI CAA burden score

The MRI CAA SVD burden score is an ordinal score of four MRI CAA biomarkers: lobar CMBs, cortical superficial siderosis, enlarged perivascular spaces in the centrum semiovale and WMH.[214] It provides a "total SVD burden" score in CAA. It was developed in a retrospective single-centre cross-sectional study 105 participants with pathologic evidence of CAA and 1.5T or 3T brain MRI, 54 of whom had a symptomatic lobar ICH. The MRI scans were assessed by trained observers using the definitions for SVD biomarkers set out in STRIVE,[77] and masked to the clinical and histopathological information. The scoring system was pre-specified, based on evidence from cross-sectional and longitudinal studies in CAA. Points were awarded as follows:

- Lobar CMBs
    - 2-4 lobar CMBs = 1 point
    - ≥5 lobar CMBs = 2 points
- Cortical superficial siderosis
    - Focal cortical superficial siderosis = 1 point
    - Disseminated cortical superficial siderosis = 2 points
- Moderate to severe enlarged perivascular spaces in the centrum semiovale (grade 3 or 4 – Section 0) = 1 point
- Periventricular WMH Fazekas 3 and/or deep WMG Fazekas 2-3 (Section 0) = 1 point.

The combined 7-point ordinal score, therefore, assesses the global burden of SVDs from a minimum score of 0 to a maximum score of 6.

The severity of CAA-associated vasculopathic changes and CAA presentation with symptomatic ICH were independently associated with the total MRI CAA SVD burden score in the development study.[214]

The MRI CAA burden score was developed in symptomatic participants with pathologically proven CAA, however it was designed to assess the total CAA SVD burden in patients without pathological CAA assessment, and showed an independent association with the severity of pathological CAA-associated features. Therefore, I applied it in all participants with adequate MRI brain scans, regardless of ICH location or Boston criteria classification.

Image analysis training

I have completed four years of Clinical Radiology training, including one year of neuroradiology, and am a Fellow of the Royal College of Radiologist UK. Through my clinical radiology training, I am familiar with the range of SVD MRI biomarkers. In addition, I reviewed training materials for acute ischaemic stroke classification, WMH, atrophy, enlarged perivascular spaces and CMBs before rating the research MRI scans.[215]

MRI rating

I performed the visual ratings of the clinical and research brain MRI scans using Carestream Vue PACS version 11.3.2, Carestream Health, Inc, USA. I performed all assessments masked to clinical, genetic, CT, histopathological and outcome data.

2.3 APOE genotyping

APOE genotyping was performed using either peripheral blood or cerebellar brain tissue.

2.3.1 Peripheral blood samples

Two peripheral blood samples were taken by members of the RUSH research team using nine-millilitre ethylene-diamine-tetra-acetic acid (EDTA) tubes. Samples were anonymised with a unique four-digit study ID number and the participant's date of birth and sent to the Edinburgh Wellcome Trust Clinical Research Facility (WTCRF, Western General Hospital, Edinburgh) for storage and future genotyping.

2.3.2 Brain tissue samples

Many participants died before peripheral blood samples could be taken. Therefore cerebellar vermis brain tissue samples from the LINCHPIN brain bank were also sent to the Edinburgh WTCRF for APOE genotyping. The details of LINCHPIN research brain autopsies are described in section 2.4.

2.3.3 DNA extraction

WTCRF laboratory staff extracted DNA from whole blood using a Nucleon Kit (GenProbe) with the BACC3 protocol. DNA samples were re-suspended in 1 ml TE buffer pH 7.5 (10mM Tris-Cl pH 7.5, 1mM EDTA pH 8.0). The yield of DNA was measured using picogreen and normalised to 10ng/µl before genotyping.

WTCRF laboratory staff extracted DNA from fresh-frozen cerebellar brain tissue by homogenising using buffer ATL with proteinase K and incubating at 56ºC on a thermomixer at 1000 rpm then isolated using Qiagen DNeasy blood and tissue kit. DNA samples were resuspended in 200 µl of Qiagen elution buffer and normalised to 10ng/µl before genotyping.

WTCRF laboratory staff extracted DNA from formalin fixed paraffin embedded cerebellar tissue brain tissue using the Covaris E220 Focused Ultra Sonicator and the truXTRAC FFPE DNA kit, following the genomic DNA extraction protocol. 20 µm tissue scrolls were deparaffinised by sonication for 2 x 5 minute periods before overnight incubation on a thermomixer at 56ºC with proteinase K. Crosslinking was reversed by incubation at 80ºC for 1 hour before purification in spin columns, and elution in 50 µL of Covaris Buffer BE (5mM Tris HCl pH 8.5).

2.3.4 APOE genotyping

WTCRF laboratory staff determined genotypes for two APOE single-nucleotide polymorphisms (rs429358 and rs7412) using TaqMan single-nucleotide polymorphism genotyping assays (Applied Biosystems, Foster City, CA) on a ThermoFisher QuantStudio 12K Flex Real-Time PCR System

instrument with QuantStudio 12K Flex Software or Taqman Genotyper Software v1.3.

WTCRF laboratory staff were masked to clinical, CT, MRI and pathological features.

2.3.5 APOE genotype definition

I used the two APOE single-nucleotide polymorphisms (rs429358 and rs7412) to determine alleles (Table 2.5).[216] APOE single-nucleotide polymorphisms (rs429358 (C;T) and rs7412 (C;T)) are ambiguous for ε2/ε4 and ε1/ε3 genotypes. In such cases, I classified the genotype as ε2/ε4 given that ε1/ε3 is very uncommon. I classified APOE genotype as APOEε2 possession if participants had at least one ε2 allele and APOEε4 possession if they had at least one ε4 allele.

Table 2.5 APOE genotypes and corresponding alleles

rs429358	rs7412	Genotype
C;C	T;T	Apo-ε1/ε1
C;T	T;T	Apo-ε1/ε2
C;T	C;T	Apo-ε2/ε4
C;C	C;T	Apo-ε1/ε4
T;T	T;T	Apo-ε2/ε2
T;T	C;T	Apo-ε2/ε3
T;T	C;C	Apo-ε3/ε3
C;T	C;C	Apo-ε3/ε4
C;C	C;C	Apo-ε4/ε4

APOE = apolipoprotein E.

2.4 LINCHPIN research brain autopsy

LINCHPIN research autopsies were performed by one neuropathologist (Professor C Smith) according to a standard operating procedure.[217] The maximum interval from death to autopsy was five days.

The cerebral hemispheres were sectioned in the coronal plane at 1 cm intervals, the first slice taken through the mammillary bodies. The cerebellum was sectioned in the sagittal plane and the brainstem axially. Tissue samples approximately 20 x 20 x 10 mm were taken from each cerebral hemisphere from the frontal parasagittal cortex (BA9); Broca's area (BA44/45); temporal tip (BA38); caudate nucleus; basal ganglia; hippocampus; thalamus; frontal, temporal, parietal and occipital white matter; cerebellum; pons and medulla. Samples were bisected in the coronal plane, one block fixed in 10% unbuffered formalin for standard histological processing and the other frozen in nitrogen vapour at -150ºC.

2.4.1 SVDs assessment

2.4.1.1 CAA

CAA was sought in the cerebral and cerebellar lobe samples using immunohistochemistry with a monoclonal mouse antibody to human beta-amyloid, (Clone 6F/3D, Dako, Copenhagen) at a concentration of 1:100.

Professor C Smith rated features of CAA in all cerebral and cerebellar lobes using a consensus rating scale.[36] This rates the presence and severity of parenchymal and meningeal CAA (0–3); the presence of capillary CAA (0 or 1), and vasculopathy (0–2) in each location (Table 2.6).

2.4.1.2 Non-CAA SVD

Two neuropathologists (Professor C Smith and Dr C Humphreys) rated the presence and severity of non-CAA SVD (or "other SVD") features in the left cerebral hemisphere (frontal, central, periventricular and occipital white matter, basal ganglia at level of mammillary bodies and thalamus at level of lateral geniculate body) only, as SVD is usually considered symmetrical,[77] using haematoxylin and eosin staining with a modified version of a published scale as follows:[218]

- None: very mild, occasional arteriolosclerosis without media splitting or luminal narrowing
- Mild: widespread mild or focal moderate arteriolosclerosis
- Moderate: widespread moderate or focal severe arteriolosclerosis, with splitting of the media and narrowing of the lumen
- Severe: widespread severe arteriolosclerosis, fibrinoid necrosis, lipohyalinosis, evidence of vascular occlusion with or without recanalization

2.4.2 Non-SVDs neurodegenerative pathologies

2.4.2.1 Alzheimer's disease pathology

β-amyloid plaques and tau were sought in all cerebral and cerebellar lobes using immunohistochemistry with a monoclonal mouse antibody to human beta-amyloid, (Clone 6F/3D, Dako, Copenhagen) at a concentration of 1:100 and a monoclonal mouse antibody to human tau, (Thermo Fisher Scientific, USA) at a concentration of 1:2500 respectively. β-amyloid plaque and neurofibrillary tangle burden were rated in all cerebral and cerebellar lobe samples by Professor C Smith using validated scales (Thal phase and Braak stage respectively).[219, 220]

The Thal phase assesses the hierarchical deposition of β-amyloid plaques in the brain in Alzheimer's disease according to five phases.[219] Phase 1 is defined by β-amyloid depoists in the frontal, parietal, temporal or occipital neocortex. Phase 2 has additional allocortical β-amyloid deposits. Phase 3 has additional β-amyloid deposits in the diencephalic nuclei and striatum, phase 4 shows additional β-amyloid in distinct brainstem nuclei (substantia nigra, red nucleus, central gray, superior and inferior collicle, inferior olivary nucleus, and intermediate reticular zone). In phase 5 there is β-amyloid deposition in the cerebellum and additional brainstem nuclei (pontine nuclei, locus coeruleus, parabrachial nuclei, reticulo-tegmental nucleus, dorsal tegmental nucleus, and oral and central raphe nuclei). Absent β-amyloid plaques were classified as Thal phase 0.

The Braak stage assesses the severity and distribution of neurofibrillary tangles in the brain in Alzheimer's disease according to six stages.[220] Stages 1 and 2 are characterised by modest or numerous neurofibrillary tangles in the cortex confined to the transentorhinal region respectively. Stage 3 is characterised by severe involvement of the superficial cellular (Pre-α) layer in both the transentorhinal and entorhinal regions, with only a few neurofibrillary tangles in the Pri-α and Pre-β layers and only modest involvement of the hippocampal formation. In stage 4 the layer Pre-α is very severely affected and there is also considerable involvement of layers Pri-α and Pre-β, plus numerous neurofibrillary tangles in the hippocampal formation. Stage 5 shows more severe involvement of the layers Pri-α, Pre-β and Pre-γ, all components of the hippocampal formation are involved and the isocortex is severely affect. Stage 6 shows the most pronounced changes with the isocortex severely affected and involvement of the extrapyramidal system with neurofibrillary tangles.

Table 2.6 Consensus histopathological rating scale for CAA and CAA-associated vasculopathy.[36]

Score	Parenchymal CAA	Meningeal CAA	Capillary CAA	Vasculopathy
0	Absent	Absent	Absent	Absent
1	Scant β-amyloid deposition	Scant β-amyloid deposition	Present	Occasional vessel
2	Some circumferential β-amyloid	Some circumferential β-amyloid		Many vessels
3	Widespread circumferential β-amyloid	Widespread circumferential β-amyloid		

CAA = cerebral amyloid angiopathy.

2.5 Discussion

2.5.1 Methodological strengths

2.5.1.1 Study design, data collection and follow-up

Both LATCH and LINCHPIN were prospective community-based inception cohort studies of ICH. Both studies used multiple overlapping sources of case ascertainment. These features help limit selection bias. Completeness of baseline clinical characteristics was high.

The cause of the ICH was based on multidisciplinary consensus between neurologists, stroke physicians and neuroradiologists following review of all relevant diagnostic information.

Both LATCH and LINCHPIN used multiple sources of follow-up information to identify relevant outcomes. A consultant neurologist with an interest in stroke adjudicated the outcomes after reviewing all relevant information. The studies have up to eight years of follow-up, and the completeness of follow-up is high (Sections 10.4.1.3 and 10.4.2.3).

2.5.1.2 Brain imaging

To limit information bias, I reformatted diagnostic non-contrast brain CT scans into standard planes, slice thickness and windowing. I systematically assessed ICH and features of SVDs on the diagnostic non-contrast brain CT scans using a standardised pro forma (Appendix 1) based on published approaches where possible.[77, 190, 191, 193-196]

Research brain MRI scans were acquired using standardised parameters on one of two MRI scanners. I systematically assessed ICH and SVDs features using a standardised pro forma (Appendix 2) based on published approaches where possible.[77, 89, 194, 204-206]

I performed all imaging ratings masked to clinical, genetic, pathological and outcome data.

2.5.1.3 Research brain autopsy

Research brain autopsies were performed according to a standard operating procedure and included extensive tissue sampling. CAA, non-CAA SVDs and

Alzheimer's disease pathology were assessed by neuropathologists using published scales[36, 218-220] masked to clinical, imaging and genetic data.

2.5.2 Methodological weaknesses

Despite using a community-based approach, the sample size of the LINCHPIN study was modest, particularly for the research brain MRI (n=157) and brain autopsy components (n=126) (Figure 3.3). There was also selection bias for the research brain MRI and brain autopsy components as not all participants underwent these tests (Sections 3.4.2.3 and 4.4.2.2). However, this was the largest sample that could be achieved over six years using a methodologically rigorous approach, during which all eligible patients, where appropriate, were invited to consent to the LINCHPIN study.

It was difficult to collect follow-up data on blood pressure control and antithrombotic drug use after hospital discharge. These factors can influence the occurrence of vascular outcomes, such as recurrent ICH and incident ischaemic stroke.[158, 159]

Not all LATCH patients or LINCHPIN participants underwent vascular imaging or delayed MRI as part of their clinical workup. Therefore, some patients and participants classified as SVD-associated ICH may have had an undetected macrovascular or neoplastic cause for their ICH. However, this approach reflects clinical practice in the UK, making the results generalisable to routine clinical practice. There were no instances of ICHs related to moyamoya disease, vasculitis or reversible cerebral vasoconstriction syndrome identified during the clinical workup of patients in LATCH, which may relate to the rarity of these conditions or misidentification.

I used soft landmarks, such as the posterior surface of the brainstem and the fastigium of the fourth ventricle, to realign the diagnostic non-contrast brain CT scans. These can be distorted by mass effect related to brainstem or cerebellar ICHs, potentially affecting the orientation of the realigned planes. However, the effect of this in my studies is limited as brainstem or cerebellar ICHs occurred in a minority of participants, and most of these ICHs were small, without significant mass effect on the above landmarks. Approaches

using bony landmarks for defining planes, such as the floor of the anterior cranial fossa and posterior margin of the clivus, also vary between people and therefore would also result in between participant variation in the realigned planes.

As discussed in Section 2.2.3.3, there is ambiguity over the implementation of the Boston criteria.

Firstly, the relevance of cerebellar haematomas and CMBs in the original and modified Boston criteria is unclear. I considered cerebellar haematomas and CMBs as compatible haemorrhagic foci for both probable CAA and possible CAA when using the original and modified Boston criteria. I did this because there is increasing evidence that superficial cerebellar haemorrhages are associated with clinically diagnosed and pathologically verified CAA.[221-226]

The authors of the Boston criteria recently published clarification on the implementation of the two sets of criteria. They state “Cerebellar hemorrhages can result from either CAA or hypertensive arteriopathy and are therefore not counted by the Boston criteria, either in favor or against a probable CAA diagnosis”.[167] However, this directly contradicts the published criteria, which state that cerebellar haemorrhages are permitted in the multiple haemorrhages criterion of probable CAA in both the original and modified Boston criteria. Furthermore, a recent commentary cited a personal communication from Dr Greenberg, co-author of the Boston criteria, explaining that a primary cerebellar ICH and two or more microbleeds or one microbleed and cortical superficial siderosis would be classified as probable CAA.[227] It, therefore, remains unclear whether cerebellar haemorrhages should be considered in the criteria or not.

Secondly, it is unclear whether the proximity of cortical superficial siderosis to ICHs needs to be taken into account in the modified Boston criteria. I considered any focus of cortical superficial siderosis as a compatible haemorrhagic focus for probable or possible CAA in the modified Boston criteria as there was no location requirement of the cortical superficial

siderosis described in these criteria.[110] However, the recent article from the Boston criteria authors states that cortical superficial siderosis “near or directly connected to ICHs that have ruptured into the subarachnoid space” are not counted as separate haemorrhages.[167] What constitutes “near” is not defined.

Different interpretations of the original and modified Boston criteria will lead to different study findings. Further clarification from the Boston criteria authors on their implementation is therefore desirable, and will hopefully be addressed in the next iteration of the criteria.[228]

2.5.3 Relevance to later chapters

I used the data from LATCH in a cross-sectional analysis of the clinical and CT features of ICH (Chapter 3) and to assess the prognostic value of CT SVD biomarkers for the risk of recurrent ICH and risk of death or disability (Chapter 10 and Chapter 11) in a community-based setting. LATCH provides an ideal opportunity to assess the utility of the modified Boston criteria, the current *in vivo* reference standard for CAA-associated ICH, in clinical practice, as well as quantifying selection bias in the LINCHPIN study (Chapter 3).

I used data from LINCHPIN in a cross-sectional analysis of the MRI and pathological features of ICH (Chapter 3 and Chapter 4) and to assess the diagnostic value of MRI and CT SVD biomarkers for identifying the SVDs underlying ICH (Chapter 5 and Chapter 7-Chapter 9) in a community-based setting. I also used data from LINCHPIN to assess the prognostic value of CT SVD biomarkers the risk of recurrent ICH (Chapter 10).

Section C. Cross-sectional studies of SVD-associated ICH

Chapter 3 Cross-sectional studies of SVD-associated ICH; baseline clinical characteristics, radiological features and APOE genotype

Chapter 4 A cross-sectional study of SVD-associated ICH; histopathological features

Chapter 3 Cross-sectional studies of SVD-associated ICH; baseline clinical characteristics, radiological features and APOE genotype

3.1 Introduction

The type of SVDs underlying SVD-associated ICH varies according to ICH location. CAA almost exclusively affects cortical and leptomeningeal vessels, whereas arteriolosclerosis can affect small blood vessels anywhere in the brain.[1] Consequently, lobar ICH can be associated with both CAA and arteriolosclerosis while non-lobar ICH is almost exclusively associated with arteriolosclerosis.[1, 153, 229] Also, APOE ε4 allele possession is associated with pathologically-proven CAA.[24] Therefore the baseline clinical characteristics, radiological features and APOE genotype in SVD-associated ICH may vary between lobar and non-lobar ICH locations because of the likely differences in underlying SVDs.

The MRI-based modified Boston criteria [110] are the current non-invasive *in vivo* reference standard for identifying CAA-associated ICH.[167] The criteria rely on the identification of haemorrhagic foci to categorise the patient as probable CAA, possible CAA or no CAA, and are most accurate when MRI with blood-sensitive sequences has been performed. The criteria showed excellent sensitivity and good specificity in the development setting.[110] However, the utility of the modified Boston criteria in clinical practice is currently uncertain as MRI may not be available, and many patients with ICH may have contraindications to MRI.

LATCH is ideal for performing observational epidemiology of SVD-associated ICH, allowing estimation of the incidence and assessment of the risk factors associated with SVD-associated ICH in a community-based study. LATCH

can also be used to assess the applicability of the modified Boston criteria in contemporary clinical practice in the UK.

The community-based LINCHPIN study, which is nested within LATCH, included standardised research MRI brain scans and DNA sampling, thereby permitting the assessment of the MRI imaging biomarkers and APOE genotype associated with SVD-associated lobar and non-lobar ICH.

3.2 Aims

I aimed to:

- Determine the incidence of first-ever SVD-associated ICH according to ICH location in a community-based cohort of ICH (LATCH).
- Assess the distribution of clinical and diagnostic non-contrast brain CT features of first-ever SVD-associated ICH according to ICH location in a community-based cohort of ICH (LATCH).
- Apply the modified Boston criteria in a community-based cohort of first-ever and recurrent SVD-associated ICH (LATCH) to assess their utility in clinical practice.
- Compare clinical and baseline ICH features between ICH patients in LATCH who did and did not consent for the LINCHPIN study, to assess selection bias.
- Assess the distribution of brain MRI SVD biomarkers in first-ever SVD-associated ICH according to ICH location in the LINCHPIN study.
- Assess the APOE genotype in first-ever SVD-associated ICH according to ICH location in the LINCHPIN study.

3.3 Methods

The study design, baseline data collection, diagnostic non-contrast brain CT and research brain MRI image acquisition and analysis, and APOE genotyping used in LATCH and LINCHPIN are described in Chapter 2.

I included consecutive adult participants (aged ≥16 years) living in the NHS Lothian Health Board region who had an ICH between 1st June 2010 and 31st May 2013 (LATCH) or 1st June 2010 and 31st May 2016 (LINCHPIN) inclusive. I excluded patients with exclusively extra-axial intracranial haemorrhage and ICH secondary to an underlying cause other than SVDs.

I determined the location of the largest ICH using CHARTS when the ICH was diagnosed by brain CT or MRI,[190] or the pathology report when the ICH was diagnosed at autopsy. I measured the volume of the largest ICH using the modified ABC/2 approach on the diagnostic brain CT or MRI.[191] I was not able to quantify ICH volume in patients diagnosed at autopsy.

I applied the modified Boston criteria[110] by reviewing all available clinical and research imaging performed in patients with an SVD-associated ICH during LATCH. For five patients there was no neuroimaging performed, instead their index ICH was diagnosed at the Procurator Fiscal autopsy. In these cases histopathological assessment for CAA was not performed. I therefore reviewed the pathology reports to quantify the number and location of ICHs to apply the modified Boston criteria.

3.3.1 Statistical analysis

I calculated 95% confidence intervals (95% CI) for crude incidence using a Poisson distribution.

I used univariable analyses to compare the frequency of clinical, diagnostic non-contrast brain CT features, research brain MRI features and APOE genotype in participants with first-ever SVD-associated lobar ICH versus non-lobar ICH using χ^2 test (or Fisher's exact test, where appropriate) for categorical variables. I used the t test or the Mann-Whitney U test for normally and non-normally distributed continuous variables respectively. I performed multivariable logistic regression to assess whether clinical and CT features were independently associated with ICH location. I pre-specified the baseline comorbidities, medications on admission, admission GCS score, ICH volume, the presence of multiple simultaneous ICHs, intraventricular haemorrhage, subarachnoid haemorrhage and subdural haemorrhage, and

CT SVD score (0 versus 1-3) to include into the multivariable model. As my conclusions were based on the pre-specified multivariable models, I did not control for multiple testing in the univariable analyses.

I compared the frequency of clinical and diagnostic non-contrast brain CT features in LATCH patients who consented to the LINCHPIN study versus those who did not using χ^2 test (or Fisher's exact test, where appropriate) for categorical variables and the t test or the Mann-Whitney U test for normally and non-normally distributed continuous variables respectively.

I performed statistical analyses using R statistical package version 3.4.4.

3.3.2 Missing data

The amount of missing data was low (Table 2.2), so I did not impute any missing data.

3.4 Results

3.4.1 LATCH

3.4.1.1 Flow of patients

There were 530 patients with a spontaneous ICH in the NHS Lothian Health Board region between 1st June 2010 and 31st May 2013 inclusive. Eighty-two patients had an ICH which was secondary to a structural cause other than SVDs. Of the 448 patients with ICH presumed related to SVDs, 419 were a first-ever ICH (Figure 3.1).

3.4.1.2 The incidence of first-ever lobar and non-lobar SVD-associated ICH in LATCH

The crude incidence of all first-ever SVD-associated ICH between 1st June 2010 and 31st May 2013 was 20.2 per 100,000 per year (95% CI 18.3 to 22.2).

The crude incidence of first-ever SVD-associated lobar ICH was 10.0 per 100,000 per year (95% CI 8.7 to 11.4) and non-lobar ICH was 10.2 per

100,000 per year (95% CI 8.8 to 11.6). The incidence of both lobar and non-lobar ICH increased with age (Table 3.1)

3.4.1.3 Locations of first-ever SVD-associated ICH in LATCH

Three hundred and eighty-two (91%) of 419 patients with first-ever SVD-associated ICH had a single index ICH, whereas 37 (9%) had multiple simultaneous index ICHs. The locations of the largest ICH are shown in Table 3.2. The largest ICH was lobar in 208 (50%) and non-lobar in 211 (50%). The frontal lobe was the most frequent ICH epicentre in lobar ICH (97 [47%]). 75% of non-lobar ICHs were supratentorial and were most frequently located in the basal ganglia (93 [44%]) or thalamus (64 [30%]). Of the 37 patients with multiple index ICHs, 22 had multiple lobar ICHs, seven multiple non-lobar ICHs and eight mixed lobar and non-lobar ICHs.

3.4.1.4 Baseline clinical features of first-ever lobar and non-lobar SVD-associated ICH in LATCH

The proportion with a pre-ICH diagnosis of hypertension was significantly higher in non-lobar ICH patients compared to those with a lobar ICH on univariable analysis, whereas pre-ICH dementia was more frequent in lobar ICH patients (Table 3.3). There were no other statistically significant univariable differences in pre-existing co-morbidities between the lobar and non-lobar ICH groups. Antiplatelet drug use at the time of ICH was significantly more common in lobar ICH compared with non-lobar patients. Lobar ICH patients had significantly larger ICH volumes. There was no statistically significant difference in admission GCS score.

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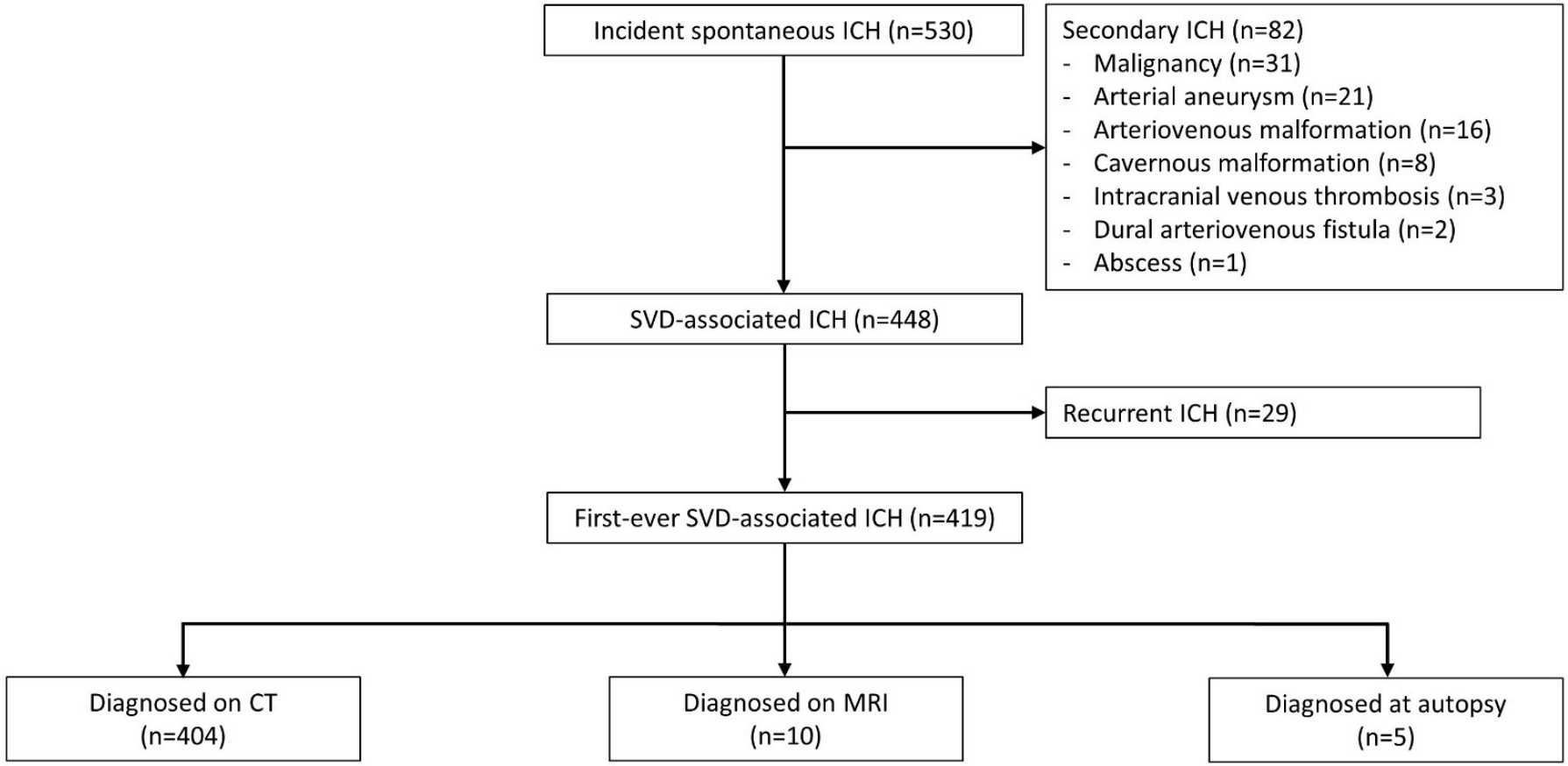
Figure 3.1 Flowchart of patients in LATCH between 1st June 2010 and 31st May 2013 inclusive



CT = computed tomography. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. MRI = magnetic resonance imaging. SVD = small vessel disease.

Table 3.1 Crude incidence of first-ever SVD-associated ICH by age and ICH location in LATCH

Age groups	**All ICH**			**Non-lobar ICH**			**Lobar ICH**		
(years)	n	Incidence	(95% CI)	n	Incidence	(95% CI)	n	Incidence	(95% CI)
16-59	65	4.2	(3.2-5.3)	37	2.4	(1.7-3.2)	28	1.8	(1.2-2.5)
60-74	112	32.9	(27.2-39.4)	58	17.0	(13.0-21.8)	54	15.9	(12.0-20.5)
75 and above	242	138.1	(121.4-156.3)	116	66.2	(54.9-79.0)	126	71.9	(60.1-85.2)
Total	419	20.2	(18.3-22.2)	211	10.2	(8.8-11.6)	208	10.0	(8.7-11.4)

Incidence expressed as n/100,000/year. ICH = Intracerebral haemorrhage. LATCH = Lothian Audit of the Treatment of Cerebral Haemorrhage. SVD = small vessel disease.

Table 3.2 Locations of first-ever SVD-associated ICHs in LATCH

ICH epicentre	Non-lobar ICH (n=211)		Lobar ICH (n=208)	
Basal ganglia	93	(44)	-	
Thalamus	64	(30)	-	
Corpus callosum	2	(1)	-	
Brainstem	18	(9)	-	
Cerebellum	34	(16)	-	
Frontal lobe	-		97	(47)
Parietal lobe	-		57	(27)
Temporal lobe	-		38	(18)
Occipital lobe	-		15	(7)
Holohemispheric	-		1	(0)

Data are n (%). ICH epicentre relates to the largest acute ICH if multiple ICHs were present at diagnosis. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. SVD = small vessel disease.

Table 3.3 Clinical characteristics of first-ever SVD-associated ICH in LATCH, stratified by ICH location

Clinical characteristics	Non-lobar ICH (n=211)		Lobar ICH (n=208)		p value
Age (years); median (IQR)	76	(65-84)	78	(69-83)	0.470
Sex					
Female	106	(50)	119	(57)	0.152
Male	105	(50)	89	(44)	
Co-morbidities*					
Hypertension	152	(72)	120	(58)	0.002
Ischaemic stroke	42	(20)	27	(13)	0.053
Transient ischaemic attack	25	(12)	20	(10)	0.450
Dementia	15	(7)	37	(18)	0.001
Diabetes	24	(11)	23	(11)	0.904
Atrial fibrillation	51	(24)	39	(19)	0.169
Myocardial infarction	18	(9)	19	(9)	0.839
Hyperlipidaemia	41	(20)	34	(16)	0.397
Smoking status					
Current	48	(23)	44	(21)	
Ex-smoker	74	(35)	73	(35)	0.941
Never	89	(42)	89	(43)	
Pre-ICH modified Rankin scale					
0	71	(34)	68	(33)	
1	39	(19)	44	(21)	
2	48	(23)	44	(21)	
3	42	(20)	40	(19)	
4	7	(3)	8	(4)	
5	0	(0)	3	(1)	
Pre-ICH modified Rankin scale; median (IQR)	2	(1-3)	2	(1-3)	0.814
Medications on admission					
Antiplatelet drug(s)	79	(37)	98	(47)	0.045
Anticoagulant drug(s)	31	(15)	25	(12)	0.421
Antihypertensive drug(s)	109	(52)	93	(45)	0.155
Admission GCS; median (IQR) †	14	(10-15)	14	(10-15)	0.422
ICH volume of the largest haematoma (ml); median (IQR) ‡	12	(4-29)	37	(12-85)	<0.001

Data are n (%) or median (IQR). * missing for 1 patient. † missing for4 patients. ‡ missing for 5 patients diagnosed by autopsy. GCS = Glasgow coma scale. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. SVD = small vessel disease.

3.4.1.5 Diagnostic brain CT scan features of first-ever lobar and non-lobar SVD-associated ICH in LATCH

Four hundred and four patients with first-ever SVD-associated ICH during LATCH were diagnosed by non-contrast brain CT (Figure 3.1). The median time between ICH onset and diagnostic brain CT was 0 days (IQR 0-1 days).

The diagnostic non-contrast brain CT features of these patients are summarised in Table 3.4. Intraventricular haemorrhage was significantly more common in the non-lobar ICH group, whereas subarachnoid haemorrhage, subdural haemorrhage and finger-like projections were significantly more frequent in lobar ICH on univariable testing. The non-lobar ICH group had significantly more frequent old vascular lesions and more severe CT SVD score compared with lobar ICH.

3.4.1.6 Multivariable analysis of baseline clinical features and diagnostic brain CT scan features of first-ever lobar and non-lobar SVD-associated ICH in LATCH

Multivariable analysis of patients with first-ever SVD-associated ICH diagnosed by CT showed no independent association between pre-existing co-morbidities and ICH location, including hypertension and dementia (Table 3.5). ICH volume was significantly higher in patients with a lobar ICH compared with non-lobar ICH, while there was a borderline association between lobar ICH and higher admission GCS. Multiple simultaneous acute ICHs, subarachnoid haemorrhage and subdural haemorrhage were independently associated with lobar ICH, whereas intraventricular haemorrhage was associated with non-lobar ICH.

3.4.1.7 Application of the modified Boston criteria during LATCH

One hundred and twenty-seven patients out of 448 with SVD-associated ICH had MRI performed (28%) during routine clinical practice to diagnose or further assess their ICH, or as part of the LINCHPIN study (Figure 3.2). Of these, one hundred and twelve patients had MRI with blood-sensitive sequences, while no blood-sensitive sequences were performed in the other 15 patients with brain MRI. Three hundred and sixteen patients had a

diagnostic brain CT scan without any clinical or research MRI performed (71%). Five patients had their ICH diagnosed at autopsy (1%).

Patients who underwent a clinical or research MRI were significantly younger than those who did not have MRI on univariable testing, with significantly less frequent pre-ICH ischaemic stroke, dementia and myocardial infarction, and less pre-ICH disability (Table 3.6). Those who had an MRI had significantly higher admission GCS, smaller ICH volume and less frequent intraventricular haemorrhage.

In total, 72 out of 448 patients with SVD-associated ICH were classified as probable CAA (16%), 166 as possible CAA (37%) and 210 as no CAA (47%) (Table 3.7). The proportion classified as probable CAA was highest in patients who underwent MRI with blood-sensitive imaging.

Table 3.4 Diagnostic non-contrast brain CT characteristics of first-ever SVD-associated ICH in LATCH, stratified by ICH location

Non-contrast brain CT characteristics	**Non-lobar ICH (n=205)**		**Lobar ICH (n=199)**		**p value**
Multiple simultaneous acute ICHs	14	(7)	23	(12)	0.099
Intraventricular haemorrhage	113	(55)	76	(38)	0.001
Subarachnoid haemorrhage	31	(15)	145	(73)	<0.001
Subdural haemorrhage	1	(1)	41	(31)	<0.001
Finger-like projections	1	(1)	43	(22)	<0.001
Old vascular lesion	99	(48)	74	(37)	0.024
Number of lacunes; median (IQR)	0	(0-1)	0	(0-0)	<0.001
Anterior white matter lucencies					
0	37	(18)	42	(21)	
1	99	(48)	105	(53)	0.247
2	69	(34)	52	(26)	
Posterior white matter lucencies					
0	63	(31)	61	(31)	
1	46	(22)	48	(24)	0.914
2	96	(47)	90	(45)	
Central atrophy					
0	39	(19)	63	(32)	
1	118	(58)	123	(62)	<0.001
2	48	(23)	13	(7)	
Cortical atrophy					
0	51	(25)	55	(28)	
1	104	(51)	106	(53)	0.424
2	50	(24)	38	(19)	
CT SVD score					
0	59	(29)	78	(39)	
1	75	(37)	80	(40)	0.006
2	55	(27)	36	(18)	
3	16	(8)	5	(3)	
CT SVD score					
0	59	(29)	78	(39)	0.027
1, 2 or 3	146	(71)	121	(61)	

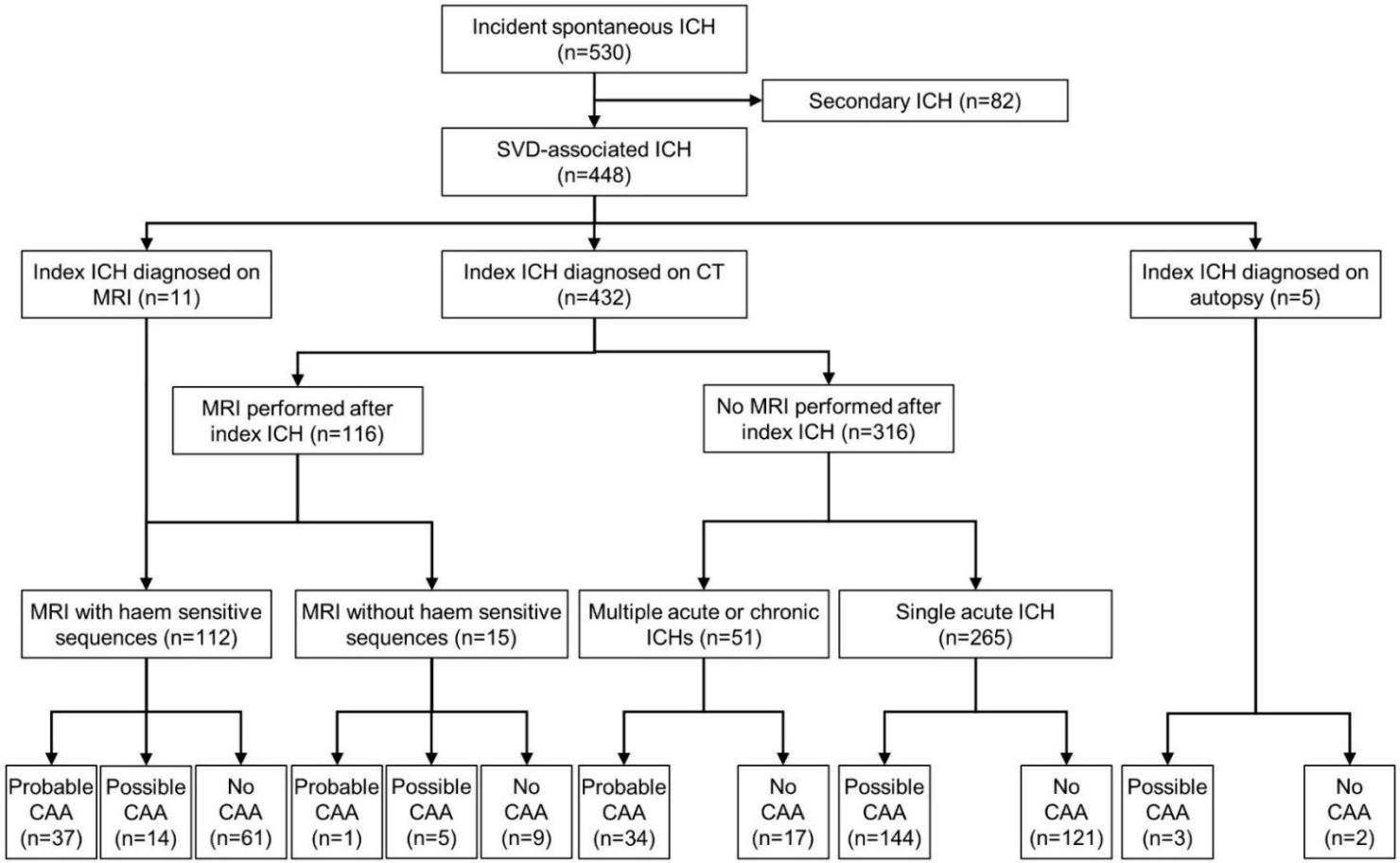
Data are n (%) or median (IQR). CT = computed tomography. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. SVD = small vessel disease.

Table 3.5 Multivariable logistic regression model of baseline clinical and diagnostic non-contrast brain CT features associated with first-ever SVD-associated lobar ICH versus non-lobar ICH in LATCH

Variable	β coefficient (SE)		Odds ratio (95% CI)		p value
Intercept	-2.56	(1.31)			0.051
Age (per year increase)	0.01	(0.01)	1.01	(0.99-1.04)	0.336
Male sex	-0.11	(0.31)	0.90	(0.49-1.64)	0.727
Hypertension	-0.71	(0.39)	0.49	(0.23-1.04)	0.066
Ischaemic stroke	-0.10	(0.44)	0.90	(0.37-2.12)	0.817
Transient ischaemic attack	-0.97	(0.50)	0.38	(0.14-1.00)	0.054
Dementia	0.73	(0.50)	2.08	(0.79-5.70)	0.145
Diabetes	0.21	(0.51)	1.23	(0.44-3.27)	0.682
Atrial fibrillation	-0.25	(0.45)	0.78	(0.31-1.86)	0.572
Myocardial infarction	0.05	(0.58)	0.95	(0.30-2.88)	0.925
Hyperlipidaemia	-0.66	(0.41)	0.52	(0.23-1.14)	0.108
Medications on admission					
Antiplatelet drug(s)	0.56	(0.38)	1.76	(0.84-3.74)	0.138
Anticoagulant drug(s)	-0.22	(0.52)	0.81	(0.29-2.23)	0.679
Antihypertensive drug(s)	-0.04	(0.39)	0.97	(0.45-2.10)	0.929
Admission GCS (per unit increase)	0.09	(0.05)	1.10	(1.00-1.21)	0.056
ICH volume (per ml increase)	0.02	(0.01)	1.02	(1.01-1.04)	<0.001
Multiple simultaneous acute ICHs	1.05	(0.50)	2.86	(1.07-7.72)	0.036
Intraventricular haemorrhage	-1.68	(0.38)	0.19	(0.09-0.38)	<0.001
Subarachnoid haemorrhage	2.43	(0.33)	11.36	(6.05-22.32)	<0.001
Subdural haemorrhage	2.75	(1.10)	15.64	(2.67-304.2)	0.012
CT SVD score 1-3 versus 0	-0.54	(0.33)	0.58	(0.31-1.11)	0.099

1 patient excluded due to missing data. CT = Computed tomography. GCS = Glasgow coma scale. ICH = intracerebral haemorrhage. LATCH = Lothian au dit of the treatment of Cerebral haemorrhage. SVD = small vessel disease.

Figure 3.2 Modified Boston criteria classification of SVD-associated ICH during LATCH



CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. MRI = magnetic resonance imaging. SVD = small vessel disease.

Table 3.6 Clinical and non-contrast brain CT features of LATCH patients with SVD-associated ICH who did and did not undergo brain MRI.

Characteristics	No MRI (n=321)		MRI (n=127)		p value
Age (years); median (IQR)	79	(71-85)	71	(58-78)	<0.001
Sex					
Female	175	(55)	60	(47)	0.165
Male	146	(45)	67	(53)	
Co-morbidities*					
Hypertension	222	(69)	74	(58)	0.025
Ischaemic stroke	64	(20)	9	(7)	0.001
Transient ischaemic attack	43	(13)	12	(9)	0.247
Dementia	57	(18)	3	(2)	<0.001
Diabetes	41	(13)	10	(8)	0.139
Atrial fibrillation	76	(24)	21	(17)	0.095
Myocardial infarction	35	(11)	4	(3)	0.009
Hyperlipidaemia	64	(20)	23	(18)	0.649
Smoking status					
Current	71	(22)	24	(19)	
Ex-smoker	118	(37)	43	(34)	0.470
Never	129	(41)	59	(47)	
Pre-ICH modified Rankin scale					
0	67	(21)	74	(58)	
1	66	(21)	23	(18)	
2	82	(26)	18	(14)	<0.001
3	79	(25)	10	(8)	
4	17	(5)	2	(2)	
5	5	(2)	0	(0)	
Pre-ICH modified Rankin scale; median (IQR)	3	(2-4)	1	(1-2)	<0.001
Medications on admission					
Antiplatelet drug(s)	146	(46)	39	(31)	0.004
Anticoagulant drug(s)	44	(14)	14	(11)	0.446
Antihypertensive drug(s)	171	(53)	48	(38)	0.003
Admission GCS; median (IQR) †	12	(8-14)	15	(14-15)	<0.001
Multiple simultaneous acute ICHs	34	(11)	5	(4)	0.038
ICH volume of the largest haematoma (ml); median (IQR) ‡	29	(9-70)	9	(3-18)	<0.001
Location of largest ICH					
Lobar	156	(49)	70	(55)	0.214
Non-lobar	165	(51)	57	(45)	
Intraventricular haemorrhage	179	(56)	25	(20)	<0.001
Subarachnoid haemorrhage	148	(46)	47	(37)	0.080
Subdural haemorrhage	37	(12)	9	(7)	0.163

Data are n (%) or median (IQR). * missing for 1 patient. † missing for4 patients. ‡ missing for 5 patients diagnosed by autopsy. GCS = Glasgow coma scale. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. SVD = small vessel disease.

Table 3.7 Modified Boston criteria classifications in SVD-associated ICH during LATCH

Modified Boston criteria classification	MRI with blood-sensitive imaging (n=112)			MRI without blood-sensitive imaging (n=15)			CT only (n=316)			Autopsy only (n=5)			Total (n=448)		
Probable CAA	37	(33)	[25-42]	1	(7)	[1-30]	34	(11)	[8-15]	0	(0)	[0-43]	72	(16)	[13-20]
Possible CAA	14	(13)	[8-20]	5	(33)	[15-58]	144	(46)	[40-51]	3	(60)	[23-88]	166	(37)	[33-42]
No CAA	61	(54)	[45-63]	9	(60)	[36-80]	138	(44)	[38-49]	2	(40)	[12-77]	210	(47)	[42-52]

Data are n (%) [95% CI]. CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. MRI = magnetic resonance imaging. SVD = small vessel disease.

3.4.2 LINCHPIN

3.4.2.1 Flow of participants

There were 612 patients with a spontaneous ICH presumed related to SVDs between 1st June 2010 and 31st May 2016. Three hundred fifty consented to the LINCHPIN study, of whom 337 presented with a first-ever ICH (Figure 3.3).

3.4.2.2 Comparison of LATCH patients with first-ever SVD-associated ICH who did and did not consent to any part of the LINCHPIN study

Between 1st June 2010 and 31st May 2013 inclusive, 187 out of 448 LATCH patients with SVD-associated ICH consented to the LINCHPIN study, of whom 178 had a first-ever SVD-associated ICH (Figure 3.4).

The age and sex distribution were similar between those who did and did not consent to any part of the LINCHPIN study on univariable testing (Table 3.18). The proportion of patients with a previous history of ischaemic stroke or myocardial infarction was significantly lower in LINCHPIN consenters versus non-consenters. The frequency of other baseline co-morbidities was similar between the groups. Pre-ICH level of functioning was significantly better in LINCHPIN consenters. Admission GCS scores were significantly higher and intraventricular haemorrhage was significantly less frequent in those who consented to LINCHPIN. ICH volume tended to be smaller in consenters, but this did not reach statistical significance (Table 3.9).

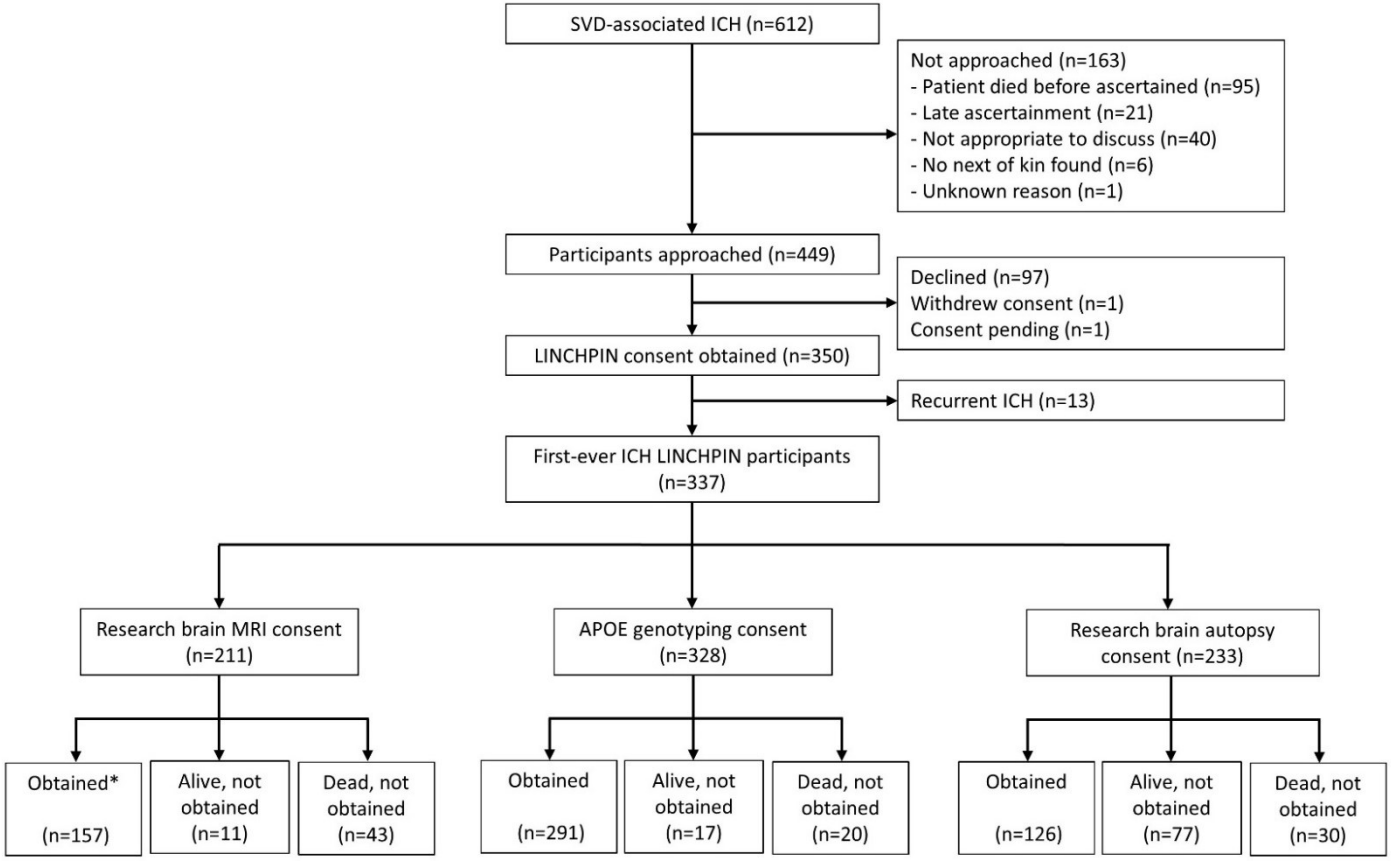
3.4.2.3 Research brain MRI

Comparison of LATCH patients with first-ever SVD-associated ICH who did and did not undergo research brain MRI as part of the LINCHPIN study

Eighty-three LATCH patients with first-ever SVD-associated ICH had a research brain MRI scan as part of LINCHPIN (Figure 3.4). Participants who had a research brain MRI were significantly younger and less likely to have a pre-ICH history of ischaemic stroke, dementia, diabetes or myocardial infarction than the rest of the patients with SVD-associated ICH in LATCH (Table 3.10). Also, those undergoing a research MRI had significantly better

pre-ICH functioning, higher admission GCS, smaller ICH volumes, were less likely to have intraventricular haemorrhage and had a lower CT SVD score (Table 3.11).

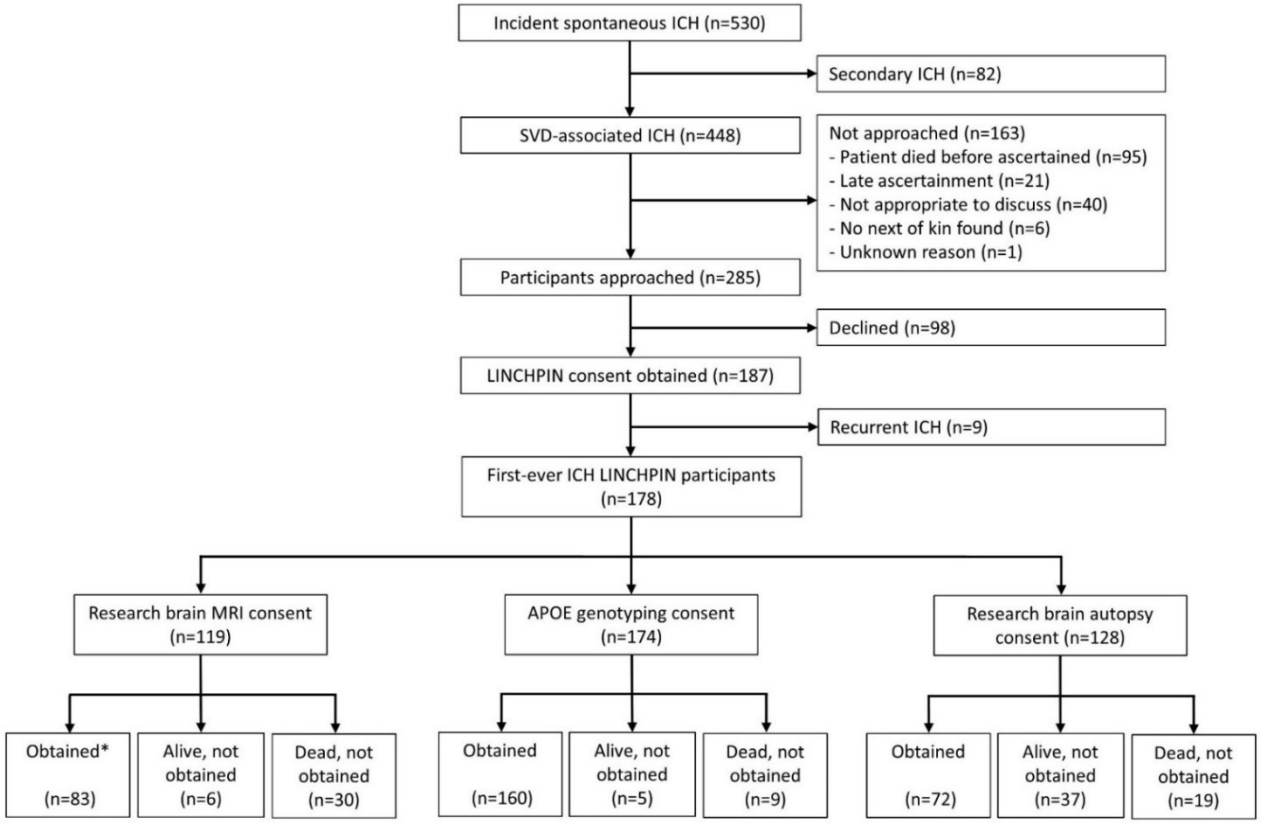
Figure 3.3 Flowchart of participants in the LINCHPIN study between 1st June 2010 and 31st May 2016 inclusive



* Two MRIs obtained but poor quality (too much movement) or no T2* sequence obtained (claustrophobia).

APOE = apolipoprotein E. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. MRI = magnetic resonance imaging. SVD = small vessel disease.

Figure 3.4 Flowchart of LATCH patients recruited into the LINCHPIN study between 1st June 2010 until 31st May 2013 inclusive



* Two MRIs obtained but poor quality (too much movement) or no T2*-weighted GRE sequence obtained due to claustrophobia. APOE = apolipoprotein E. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. MRI = magnetic resonance imaging. SVD = small vessel disease.

Table 3.8 Baseline features of LATCH patients with first-ever SVD-associated ICH who consented to the LINCHPIN study versus those who did not

Characteristics	Consented to LINCHPIN (n=178)		Did not consent to LINCHPIN (n=241)		p value
Age (years); median (IQR)	77	(67-83)	77	(67-83)	0.742
Sex					
Female	93	(52)	132	(55)	0.608
Male	85	(48)	109	(45)	
Co-morbidities*					
Hypertension	111	(62)	161	(67)	0.316
Ischaemic stroke	21	(12)	48	(20)	0.026
Transient ischaemic attack	21	(12)	24	(10)	0.558
Dementia	21	(12)	31	(13)	0.744
Diabetes	14	(8)	33	(14)	0.060
Atrial fibrillation	34	(19)	56	(23)	0.298
Myocardial infarction	9	(5)	28	(12)	0.019
Hyperlipidaemia	29	(16)	46	(19)	0.449
Smoking status					
Current	36	(20)	56	(23)	
Ex-smoker	57	(32)	90	(38)	0.196
Never	85	(48)	93	(39)	
Pre-ICH modified Rankin scale					
0	77	(44)	62	(26)	
1	29	(16)	54	(23)	
2	31	(18)	61	(26)	
3	32	(18)	50	(21)	
4	6	(3)	9	(4)	
5	2	(1)	1	(0)	
Pre-ICH modified Rankin scale; median (IQR)	2	(1-3)	3	(1-4)	0.007
Medications on admission					
Antiplatelet drug(s)	75	(41)	102	(42)	0.969
Anticoagulant drug(s)	22	(12)	34	(14)	0.603
Antihypertensive drug(s)	83	(47)	119	(49)	0.578
Admission GCS score; median (IQR) †	14	(11-15)	13	(7-15)	<0.001

Data are n (%) or median (IQR). * missing for 1 patient. † missing for 4 patients. GCS = Glasgow coma scale. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. SVD = small vessel disease.

Table 3.9 ICH location and diagnostic non-contrast brain CT features in LATCH patients with first-ever SVD-associated ICH who consented to the LINCHPIN study versus those who did not

Non-contrast brain CT characteristics	**Consented to LINCHPIN (n=178)**		**Did not consent to LINCHPIN (n=241)**		**p value**
ICH location					
Lobar	92	(52)	116	(48)	0.472
Non-lobar	86	(48)	125	(52)	
ICH volume; median (IQR) ‡	17	(5-40)	22	(6-62)	0.054
Intraventricular haemorrhage	66	(37)	125	(52)	0.003
Subarachnoid haemorrhage	78	(44)	101	(42)	0.696
Subdural haemorrhage	17	(10)	25	(10)	0.782
CT SVD score¥					
0	62	(37)	75	(32)	0.754
1	65	(38)	90	(39)	
2	35	(21)	56	(24)	
3	8	(5)	13	(6)	

Data are n (%) or median (IQR). ‡ missing for 5 patients diagnosed by autopsy. ¥ missing for 15 patients without a diagnostic CT. CT = computed tomography. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. SVD = small vessel disease.

Table 3.10 Baseline clinical features in first-ever SVD-associated ICH LATCH patients who had a LINCHPIN research brain MRI performed versus those who did not

Characteristics	Research MRI obtained (n=83)		No research MRI obtained (n=336)		p value
Age (years); median (IQR)	73	(61-79)	78	(68-84)	<0.001
Sex					
Female	43	(52)	182	(54)	0.699
Male	40	(48)	154	(46)	
Co-morbidities*					
Hypertension	47	(57)	225	(67)	0.071
Ischaemic stroke	5	(6)	64	(19)	0.004
Transient ischaemic attack	5	(6)	40	(12)	0.120
Dementia	1	(1)	51	(15)	0.001
Diabetes	4	(5)	43	(13)	0.038
Atrial fibrillation	12	(15)	78	(23)	0.080
Myocardial infarction	2	(2)	35	(10)	0.021
Hyperlipidaemia	14	(17)	61	(18)	0.776
Smoking status					
Current	21	(25)	71	(21)	
Ex-smoker	26	(31)	121	(36)	0.620
Never	36	(43)	142	(43)	
Pre-ICH modified Rankin scale					
0	57	(69)	82	(25)	
1	10	(12)	73	(22)	
2	11	(13)	81	(25)	
3	5	(6)	77	(23)	
4	0	(0)	15	(5)	
5	0	(0)	3	(1)	
Pre-ICH modified Rankin scale; median (IQR)	1	(1-2)	3	(2-4)	<0.001
Medications on admission					
Antiplatelet drug(s)	24	(29)	153	(46)	0.006
Anticoagulant drug(s)	10	(12)	46	(14)	0.694
Antihypertensive drug(s)	33	(40)	169	(50)	0.085
Admission GCS score; median (IQR) †	15	(14-15)	13	(9-15)	<0.001

Data are n (%) or median (IQR). * missing for 1 patient. † missing for4 patients. GCS = Glasgow coma scale. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. MRI = magnetic resonance imaging. SVD = small vessel disease.

Table 3.11 ICH location and diagnostic non-contrast brain CT features in first-ever SVD-associated ICH LATCH patients who had a LINCHPIN research brain MRI performed versus those who did not

	Research MRI obtained (n=83)	No research MRI obtained (n=336)	p value
ICH location			
Lobar	46 (55)	162 (48)	0.240
Non-lobar	37 (45)	174 (52)	
ICH volume; median (IQR) ‡	9 (3-20)	24 (7-63)	<0.001
Intraventricular haemorrhage	16 (19)	175 (52)	<0.001
Subarachnoid haemorrhage	31 (37)	148 (44)	0.269
Subdural haemorrhage	6 (7)	36 (11)	0.344
CT SVD score¥			
0	41 (54)	96 (29)	<0.001
1	22 (29)	133 (41)	
2	13 (17)	78 (24)	
3	0 (0)	21 (6)	

Data are n (%) or median (IQR). ‡ missing for 5 patients diagnosed by autopsy. ¥ missing for 15 patients without a diagnostic CT. CT = computed tomography. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. MRI = magnetic resonance imaging. SVD = small vessel disease.

Brain MRI scan features of first-ever lobar and non-lobar ICH in LINCHPIN

One hundred and fifty-seven LINCHPIN participants with first-ever SVD-associated ICH had a research brain MRI (Figure 3.3). I excluded two participants due to poor quality MRI scans (one had too much motion artefact to allow accurate analysis; the other had no T2*-weighted GRE sequence obtained due to claustrophobia). Eighty-one (52%) of the remaining 155 participants with research brain MRI had a non-lobar ICH and 74 (48%) had a lobar ICH. The median time between ICH onset and research brain MRI was 92 days (IQR 73-125 days).

There was no statistically significant difference in the severity of WMH, atrophy or PVS between the groups (Table 3.12). Lobar ICH participants were more likely to have cortical superficial siderosis than the non-lobar ICH

group. When present, cortical superficial siderosis was more likely to be diffuse (i.e. both adjacent to and distant from the ICH) in those with a lobar ICH. Non-lobar ICH participants had more deep CMBs whereas there was no statistically significant difference in the number of lobar, cerebellar, brainstem or total CMBs between the groups.

There was no significant difference in MRI SVD burden score[197] between the groups. The MRI CAA SVD burden score[214] was higher in lobar ICH participants.

Table 3.12 Research brain MRI characteristics of SVD-associated ICH in the LINCHPIN study, stratified by ICH location

	Non-lobar ICH (n=81)		Lobar ICH (n=74)		p value
Periventricular Fazekas score*					
0	3	(4)	2	(3)	
1	20	(25)	22	(30)	
2	30	(37)	12	(16)	
3	28	(35)	38	(51)	
Periventricular Fazekas score; median (IQR)	2	(1-3)	3	(1-3)	0.262
Deep Fazekas score					
0	8	(10)	9	(12)	
1	31	(38)	21	(28)	
2	27	(33)	32	(43)	
3	15	(19)	12	(16)	
Deep Fazekas score; median (IQR)	2	(1-2)	2	(1-2)	0.721
Central atrophy*					
1	24	(30)	13	(18)	
2	16	(20)	22	(30)	
3	20	(25)	19	(26)	
4	11	(14)	7	(10)	
5	10	(12)	9	(12)	
6	0	(0)	4	(5)	
Central atrophy; median (IQR)	3	(1-4)	3	(2-4)	0.305
Cortical atrophy*					
1	38	(47)	29	(39)	
2	20	(25)	20	(27)	
3	11	(14)	9	(12)	
4	4	(5)	4	(5)	
5	6	(7)	9	(12)	
6	2	(3)	3	(4)	
Cortical atrophy; median (IQR)	2	(1-3)	2	(1-3)	0.267
Basal ganglia PVS*					
0	0	(0)	0	(0)	
1	43	(53)	47	(64)	
2	23	(28)	18	(24)	
3	12	(15)	8	(11)	
4	3	(4)	1	(1)	
Basal ganglia PVS; median (IQR)	1	(1-2)	1	(1-2)	0.157
Centrum semiovale PVS*					
0	2	(3)	3	(4)	
1	30	(37)	20	(27)	
2	33	(41)	28	(38)	
3	15	(19)	16	(22)	
4	1	(1)	7	(10)	
Centrum semiovale PVS; median (IQR)	2	(1-2)	2	(1-3)	0.106
Multiple ICH	11	(14)	12	(16)	0.645

	Non-lobar ICH (n=81)		Lobar ICH (n=74)		p value
Cortical superficial siderosis	12	(15)	49	(66)	<0.001
Cortical superficial siderosis extent					
Focal	9	(75)	25	(49)	0.134
Diffuse	3	(25)	24	(51)	
Cortical superficial siderosis location*					
Adjacent to ICH	7	(58)	23	(47)	
Distant to ICH	3	(25)	2	(4)	0.019
Both	2	(17)	24	(49)	
Any lobar CMB	23	(28)	28	(38)	0.211
Total lobar CMBs; median (IQR)	0	(0-1)	0	(0-2)	0.192
Any deep CMB	28	(35)	12	(16)	0.009
Total deep CMBs; median (IQR)	0	(0-1)	0	(0-0)	0.005
Any cerebellar CMB	12	(15)	5	(7)	0.109
Total cerebellar CMBs; median (IQR)	0	(0-0)	0	(0-0)	0.105
Any brainstem CMB	10	(12)	3	(4)	0.063
Total brainstem CMBs; median (IQR)	0	(0-0)	0	(0-0)	0.060
Any CMB	39	(48)	32	(43)	0.540
Total CMBs; median (IQR)	0	(0-4)	0	(0-2)	0.408
MRI SVD burden score					
0	15	(19)	15	(20)	
1	18	(22)	17	(23)	
2	19	(24)	20	(27)	
3	20	(25)	20	(27)	
4	9	(11)	2	(3)	
MRI SVD burden score; median (IQR)	3	(2-4)	3	(2-4)	0.390
MRI CAA SVD burden score					
0	26	(32)	13	(18)	
1	36	(44)	19	(26)	
2	12	(15)	17	(23)	
3	3	(4)	13	(18)	
4	2	(3)	9	(12)	
5	1	(1)	1	(1)	
6	1	(1)	2	(3)	
MRI CAA SVD burden score; median (IQR)	2	(1-2)	3	(2-4)	<0.001
Modified Boston Criteria					
No CAA	72	(89)	20	(27)	<0.001
Possible CAA	3	(4)	10	(14)	
Probable CAA	6	(7)	44	(60)	

Data are n (%) or median (IQR). * Fisher's exact test. CAA = cerebral amyloid angiopathy. CMB = cerebral microbleed. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. MRI = magnetic resonance imaging. PVS = perivascular space. SVD = small vessel disease.

3.4.2.4 APOE genotype

Comparison of LATCH patients with first-ever SVD-associated ICH who did and did not undergo APOE genotyping as part of the LINCHPIN study

One hundred and sixty LATCH patients with first-ever SVD-associated ICH had APOE genotyping performed on either a peripheral blood sample or autopsy brain tissue as part of LINCHPIN (Figure 3.4). Those who had DNA obtained for APOE genotyping had a significantly lower frequency of previous ischaemic stroke or myocardial infarction, less pre-ICH disability, higher admission GCS scores and less frequent intraventricular haemorrhage than the rest of the LATCH cohort on univariable testing (Table 3.13 and Table 3.14).

APOE genotype in first-ever lobar and non-lobar ICH in LINCHPIN

Two hundred and ninety-one LINCHPIN participants had APOE genotyping performed (Figure 3.3). The genotype was undetermined in 14. Of the remaining 277 participants, 135 had a lobar ICH and 142 a non-lobar ICH.

APOE ε2 allele possession was significantly more common in lobar versus non-lobar ICH (37/135 [27%] versus 15/142 [11%] respectively, p<0.001). APOE ε4 allele possession was more frequent in lobar ICH versus non-lobar ICH, but this did not reach statistical significance on univariable analysis (52/135 [39%] versus 41/142 [29%] respectively, p=0.089). APOE ε2 possession was independently associated with lobar ICH location after adjusting for baseline co-morbidities (Table 3.15). There was a borderline statistically significant association between APOE ε4 possession with lobar ICH (OR 1.73 [95%CI 0.99-3.07]).

Table 3.13 Baseline features in first-ever SVD-associated ICH LATCH patients who had APOE genotyping performed as part of LINCHPIN versus those who did not

Characteristics	DNA for APOE genotype (n=160)		No DNA for APOE genotype (n=259)		p value
Age (years); median (IQR)	77	(68-83)	77	(67-84)	0.588
Sex					
Female	84	(52)	141	(54)	0.699
Male	76	(48)	118	(46)	
Co-morbidities*					
Hypertension	100	(63)	172	(67)	0.385
Ischaemic stroke	19	(12)	50	(19)	0.045
Transient ischaemic attack	19	(12)	26	(10)	0.564
Dementia	18	(11)	34	(13)	0.571
Diabetes	13	(8)	34	(13)	0.112
Atrial fibrillation	30	(19)	60	(23)	0.276
Myocardial infarction	7	(4)	30	(12)	0.011
Hyperlipidaemia	27	(17)	48	(19)	0.654
Smoking status					
Current	33	(21)	59	(23)	
Ex-smoker	52	(33)	95	(37)	0.393
Never	75	(47)	103	(40)	
Pre-ICH modified Rankin scale					
0	71	(45)	68	(27)	
1	26	(16)	57	(22)	
2	26	(16)	66	(26)	
3	28	(18)	54	(21)	
4	6	(4)	9	(4)	
5	2	(1)	1	(0)	
Pre-ICH modified Rankin scale; median (IQR)	2	(1-3)	3	(1-4)	0.006
Medications on admission					
Antiplatelet drug(s)	67	(42)	110	(43)	0.904
Anticoagulant drug(s)	19	(12)	37	(14)	0.481
Antihypertensive drug(s)	73	(46)	129	(50)	0.405
Admission GCS score; median (IQR) †	14	(11-15)	14	(8-15)	0.005

Data are n (%) or median (IQR). * missing for 1 patient. ∞ missing for 22 patients. † missing for4 patients. APOE = apolipoprotein E. GCS = Glasgow coma scale. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. SVD = small vessel disease.

Table 3.14 ICH location and diagnostic non-contrast brain CT features in first-ever SVD-associated ICH LATCH patients who had APOE genotyping performed as part of LINCHPIN versus those who did not

	DNA for APOE genotype (n=160)		No DNA for APOE genotype (n=259)		p value
ICH location					
Lobar	82	(51)	126	(49)	0.605
Non-lobar	78	(49)	133	(51)	
ICH volume; median (IQR) ‡	17	(5-40)	20	(6-61)	0.272
Intraventricular haemorrhage	63	(39)	128	(49)	0.045
Subarachnoid haemorrhage	71	(44)	108	(42)	0.591
Subdural haemorrhage	14	(9)	28	(11)	0.495
CT SVD score¥					
0	54	(35)	83	(33)	0.969
1	58	(38)	97	(39)	
2	33	(22)	58	(23)	
3	8	(5)	13	(5)	

Data are n (%) or median (IQR). ‡ missing for 5 patients diagnosed by autopsy. ¥ missing for 15 patients without a diagnostic CT. APOE = apolipoprotein E. CT = computed tomography. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. SVD = small vessel disease.

Table 3.15 Multivariable logistic regression model of baseline clinical features and APOE genotype associated with first-ever SVD-associated lobar ICH during the LINCHPIN study

	β coefficient (SE)		Odds ratio	(95% CI)	p value
Intercept	-2.62	(0.91)			0.004
Age (per year increase)	0.04	(0.01)	1.04	(1.02-1.07)	0.001
Male sex	-0.49	(0.27)	0.61	(0.36-1.05)	0.073
Hypertension	-0.72	(0.30)	0.49	(0.27-0.87)	0.016
Ischaemic stroke	-0.54	(0.43)	0.58	(0.25-1.33)	0.205
Transient ischaemic attack	-0.08	(0.47)	0.92	(0.37-2.31)	0.862
Dementia	0.01	(0.47)	0.99	(0.40-2.49)	0.979
Diabetes	0.21	(0.44)	1.23	(0.51-2.92)	0.642
Atrial fibrillation	0.05	(0.37)	1.05	(0.51-2.18)	0.886
Myocardial infarction	0.89	(0.53)	2.43	(0.88-7.26)	0.095
Hyperlipidaemia	-0.29	(0.39)	0.75	(0.34-1.60)	0.456
APOE ε2 allele possession	1.21	(0.37)	3.36	(1.67-7.06)	<0.001
APOE ε4 allele possession	0.55	(0.29)	1.73	(0.99-3.07)	0.055

APOE = apolipoprotein. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. SVD = small vessel disease.

3.5 Discussion

3.5.1 Main findings

- The incidence of lobar and non-lobar first-ever SVD-associated ICH was similar and increased with age.
- Baseline clinical and diagnostic CT findings in a community-based cross-sectional study of first-ever SVD-associated ICH (LATCH)
    - Pre-existing dementia was more frequent in patients with a lobar ICH, and pre-ICH hypertension more common in non-lobar ICH patients, but these associations were not statistically significant after adjustment for other baseline clinical and non-contrast CT brain features.
    - Patients with lobar ICH had statistically larger ICH volume and more frequent subarachnoid and subdural haemorrhage, while non-lobar ICH patients had more frequent intraventricular

haemorrhage after adjustment for other baseline clinical and non-contrast CT brain features.

- Applying the modified Boston criteria in a population-based cross-sectional study of SVD-associated ICH (LATCH)
    - Only 28% of patients underwent brain MRI as part of routine clinical practice or as part of the LINCHPIN study.
    - Those who had MRI were younger, had fewer co-morbidities, higher admission GCS scores, smaller ICH volumes and less frequent intraventricular haemorrhage compared with those who did not have an MRI.
    - Overall, 16% of patients were classified as probable CAA, 37% as possible CAA and 47% as no CAA.
    - In the 25% of patients who had MRI with blood-sensitive imaging, 33% were classified as probable CAA, 13% as possible CAA and 54% as no CAA.
- Assessment of selection bias in LINCHPIN during a population-based cross-sectional study of first-ever SVD-associated ICH (LATCH)
    - Those who consented to any part of LINCHPIN were similar to non-consenters apart from being less likely to have had a previous ischaemic stroke or myocardial infarction, and having a higher admission GCS and less frequent intraventricular haemorrhage.
    - Those who had a research brain MRI were younger than the rest of the LATCH cohort, with less frequent pre-ICH ischaemic stroke, dementia, diabetes or myocardial infarction and less severe pre-ICH disability. Admission GCS was higher in those undergoing research brain MRI, and they had smaller ICHs, less frequent intraventricular haemorrhage and less severe CT SVD score.
    - Those who had DNA obtained for APOE genotyping had less pre-ICH disability, higher admission GCS and less frequent

intraventricular haemorrhage than the rest of the LATCH cohort.

- Research MRI findings in a community-based cross-sectional study of first-ever SVD-associated ICH (LINCHPIN)
    - Cortical superficial siderosis was more frequent in lobar ICH while there were more deep CMBs in non-lobar ICH.
    - The severity of WMH, atrophy and PVS were similar between lobar and non-lobar ICH groups.
- APOE genotype in a community-based cross-sectional study of first-ever SVD-associated ICH (LINCHPIN)
    - APOE ε2 possession was independently associated with lobar ICH location while APOE ε4 allele possession showed a borderline independent association.

3.5.2 Strengths of the study

I have discussed the strengths of LATCH and the LINCHPIN study in section 2.5.1. These include prospective, community-based inception cohort study designs, low levels of missing baseline clinical data and standardised approaches to assessing brain imaging to minimise information-bias. The nesting of LINCHPIN within the community-based LATCH study allowed me to examine selection biases in different components of this study. Also, I restricted my analyses of the clinical, imaging and genetic features in SVD-associated ICH to those with a first-ever ICH to standardise the inception point.

3.5.3 Weaknesses of the study

The weaknesses of LATCH and the LINCHPIN study are discussed in section 2.5.2. The main weaknesses relate to the modest sample size and selection biases in the LINCHPIN study, particularly for those undergoing a research MRI (see section 3.5.4.4).

3.5.4 Comparison with other studies

3.5.4.1 ICH incidence

The overall incidence of first-ever SVD-associated ICH (20.2 per 100,000 person-years) was similar to the incidence rate in a systematic review of 36 population-based studies of ICH (24.6 per 100,000 person-years).[134] The incidence increased with age, which is consistent with other studies,[134] and is thought to be related to the higher incidence of CAA and hypertension with age and the use of antithrombotic drugs in this age group.[135]

The overall incidence of lobar and non-lobar ICH was similar. This relationship persisted across all age groups. Other studies have shown a higher incidence of non-lobar ICH in younger patients compared with lobar ICH.[135, 230] This difference may, in part, be driven by ethnic background, with a higher frequency of non-lobar ICH found in blacks compared to whites.[231] In contrast, the patients in LATCH were almost entirely white.

3.5.4.2 Cross-sectional clinical, imaging and genetic features in lobar versus non-lobar ICH

Hypertension was common in the LATCH cohort, and more frequent in patients with non-lobar ICH compared to those with lobar ICH (72% versus 58%). However, this difference was not statistically significant when I adjusted for other baseline clinical and diagnostic brain CT features. Hypertension is often considered a more important risk factor for non-lobar than lobar ICH.[232] A meta-analysis of studies comparing the frequency of hypertension between deep and lobar supratentorial ICH showed that while hypertension was more common in patients with deep ICH, this finding may be related to biases in study design.[141] Hypertension appears to be important in most SVD-associated ICH, regardless of ICH location, and so should not be used on its own to determine the likely underlying pathology.

CAA is thought to be associated with lobar but not non-lobar ICH.[153] Autopsy studies of community-dwelling elderly have shown that CAA is associated with dementia during life, independent of Alzheimer's disease pathology.[21, 233] Therefore, there may be an association between pre-ICH

dementia and lobar ICH. Pre-ICH dementia was more frequent in patients with a lobar ICH versus non-lobar ICH (18% versus 7%), although this did not remain significant in the multivariable analysis.

There were no other statistically significant differences in baseline clinical characteristics of patients with lobar and non-lobar ICHs.

Patients with a lobar ICH had a significantly larger ICH compared to non-lobar ICH. This is consistent with previous studies,[234-236] and is probably related in part to anatomical factors; the cerebral lobes are less confined by the skull and dura than infratentorial non-lobar regions, and less confined by surrounding brain tissue than supratentorial non-lobar regions, especially if there is brain atrophy. Other features which may contribute to larger lobar ICH include more common pre-ICH use of antiplatelet drugs[235] and more frequent APOE ε2 allele possession[236] in these patients.

The higher frequency of subarachnoid and subdural haemorrhage with lobar ICH may reflect the likely underlying type of SVDs. CAA, which involves the leptomeningeal and cortical vessels,[1, 229] is thought to be associated with lobar but not non-lobar ICH.[153] Rupture of these vessels is likely to spread into the extra-axial spaces as well as the brain parenchyma. It may also be related to the anatomy, as an ICH in the cerebral lobes is closer to the cortical surface and tends to be larger,[237] increasing the chance of haemorrhage extension into the extra-axial spaces. The higher frequency of intraventricular haemorrhage with non-lobar ICH probably reflects the proximity of these haematomas to the ventricular system.[238, 239]

The severity of WMH was similar between lobar and non-lobar ICH, which is in keeping with previous studies.[240, 241] ICHs are thought to represent late consequences of SVDs[170] and WMH occur in both CAA and arteriolosclerosis.[1, 77] Therefore, a high severity of WMH in both lobar and non-lobar ICH is not surprising.

Cortical superficial siderosis was more frequent in patients with lobar ICH compared to non-lobar ICH. This is similar to a previous study,[241] and is

likely to reflect underlying CAA affecting the cortical and leptomeningeal vessels.[1, 118, 229] The higher frequency of diffuse cortical superficial siderosis and cortical superficial siderosis distant to the ICH in the lobar ICH group suggests this association is not simply due to direct extension of lobar ICH into the subarachnoid space.

ICH and CMBs are haemorrhagic manifestations of both CAA and arteriolosclerosis.[1, 77] Previous studies have shown that the regional distribution of CMBs is associated with ICH location, [240, 242] which probably reflects the distribution of vessels involved by the underlying SVDs. In line with these studies, I found that the presence and number of deep CMBs were significantly higher in non-lobar versus lobar ICH. The presence and number of lobar CMBs were higher in the lobar ICH group, although these differences did not reach statistical significance. This may reflect the small sample size in my study. Also, the inclusion of cerebellar ICH in the non-lobar group may have increased the proportion with lobar CMBs given that cerebellar ICH can be caused by either CAA or arteriolosclerosis.[226]

Lobar ICH location was independently associated with APOE ε2 possession, and there was a borderline association with APOE ε4 allele possession. These associations are consistent with a large genetic association study,[144] and are thought to be due to the presence of CAA-associated lobar ICH. Interestingly, a dose-dependent association between APOE ε4 and pathologically proven CAA was found in a meta-analysis, however, there was no overall association found between APOE ε2 and CAA.[24]

3.5.4.3 Application of the modified Boston criteria in clinical practice

The modified Boston criteria are the non-invasive *in vivo* reference standard for identifying CAA-associated ICH and are frequently used in clinical practice.[110, 167] The criteria showed good diagnostic accuracy in the development setting. However, there are two unanswered questions regarding their diagnostic value. Firstly, the criteria have never been rigorously externally validated, meaning their true diagnostic accuracy is

uncertain. I investigated this question in chapter 5. Secondly, it is unclear how applicable these MRI-based criteria are in routine clinical practice.

We offered all suitable SVD-associated ICH patients a research brain MRI during our community-based study of ICH. These scans were fully funded and performed on a dedicated research MRI scanner (i.e. not limited by clinical resources). Even so, only 28% of patients during LATCH had a brain MRI scan. This low uptake of MRI scanning is likely to reflect the high early fatality rate coupled with contraindications to MRI and survivors being unable to tolerate MRI. The proportion of SVD-associated ICH patients able to undergo brain MRI in other settings may be lower, particularly in middle- and low-income countries where access to MRI may be more limited. Therefore, MRI-based diagnostic and prognostic criteria are likely to have limited applicability in SVD-associated ICH.

Overall, 16% of SVD-associated ICH patients in LATCH were classified as probable CAA and 37% as possible CAA on the modified Boston criteria. In comparison, 43% of participants in a hospital-based study of ICH were classified as probable CAA using MRI with blood-sensitive imaging, while 27% were classified as possible CAA.[243] These differences may reflect selection biases in the hospital-based study, which only included participants who had had a brain MRI with blood-sensitive sequences. The increased sensitivity of blood-sensitive sequences for previous haemorrhagic foci is also likely to partially explain these differences. In line with this, I found that the proportion of patients classified as probable CAA varied according to whether or not MRI with blood-sensitive imaging was performed. Among the 25% of LATCH patients with MRI and blood-sensitive imaging, 33% (95%CI 25-42%) were classified as probable CAA, while 13% (95%CI 8-20%) were classified as possible CAA. In contrast, only 10% (95%CI 8-14%) of those without blood-sensitive MRI imaging were classified as probable CAA, while 45% (95%CI 40-51%) were classified as possible CAA. A similar pattern was shown by Knudsen et al. where 50% with blood-sensitive MRI sequences were classified as probable CAA on the original Boston criteria, compared to 22% without MRI and blood-sensitive sequences.[103]

3.5.4.4 Selection bias in the LINCHPIN study

Few previous studies of SVD-associated ICH have assessed for selection bias. Therefore, it is difficult to know to which patients the results are applicable. The nesting of LINCHPIN within the community-based LATCH study allowed me to assess for selection bias.

LATCH patients who consented to LINCHPIN were largely similar to those who did not consent. The main differences were lower admission GCS and more frequent intraventricular haemorrhage in non-consenters compared with consenters. This probably relates to the high early case fatality related to these factors,[152, 244, 245] which makes the discussion of and consent for research difficult.[246]

LATCH patients who underwent a LINCHPIN research brain MRI were younger, had fewer pre-ICH co-morbidities and less pre-ICH disability, smaller ICHs and less severe CT SVD scores compared with the rest of the cohort. These differences occurred despite inviting all eligible and suitable ICH patients to consent to research MRI. They likely reflect the challenges of performing MRI in ICH, where the early fatality rate is high, and many of those who survive are disabled and unable to tolerate MRI scanning,[134] and are therefore more reflective of clinical practice.

LATCH patients who had APOE genotyping performed had less severe pre-ICH disability and less frequent intraventricular haemorrhage, which is probably related to the poorer prognosis associated with these variables.[151] Otherwise, they were largely similar to those who did not have APOE genotyping.

3.5.5 Clinical implications

The incidence of SVD-associated ICH increases dramatically with age. The overall incidence of SVD-associated ICH is therefore likely to increase as the population ages.[135, 136] The use of antithrombotic drugs in elderly patients needs careful consideration given the postulated link with the high ICH incidence.[135, 136]

Hypertension is a common co-morbidity in all SVD-associated ICH, regardless of ICH location. Preventing the development of hypertension, combined with its early detection and effective management, are important steps to reduce the incidence of ICH.

The MRI-based modified Boston criteria for CAA are likely to have more limited applicability in clinical practice than in research settings for ICH given the difficulty in performing MRI in this patient group combined with limited clinical access to MRI. The diagnostic accuracy of the modified Boston criteria is likely to be worse in those without blood-sensitive MRI sequences.

3.5.6 Future directions

Future epidemiological studies should assess the influence of age, hypertension and antithrombotic drug use on the changing incidence of ICH. Some evidence of the change in incidence and aetiology of ICH is available from cohort studies from Oxfordshire[135] and Dijon.[136] However, future studies should include patients from high, middle and low-income countries and from different ethnic backgrounds.

The risks and benefits of antithrombotic drugs in the elderly, as well as in those who have had an ICH need to be further studied given the risk of both haemorrhagic and vaso-occlusive events.[151, 247] A recent randomised controlled trial assessed the risk of recurrent ICH in SVD-associated ICH survivors who were taking an antiplatelet drug for the prevention of occlusive vascular disease at the time of ICH who were randomised to either restart or stop the antiplatelet agent. The group restarted on an antiplatelet agent actually had a borderline significantly lower risk of recurrent ICH compared with the group not restarted on an antiplatelet (adjusted hazard ratio 0·51, 95% CI 0·25–1·03; p=0·060).[160] Several other ongoing randomised controlled trials are assessing whether antithrombotic drugs result in an overall benefit in serious vascular events after ICH.[248-251]

Studies of the clinical and economic impact of the modified Boston criteria on clinical care, along with rigorous external validation of the criteria, are required to determine the clinical utility and accuracy of these criteria.

Chapter 4 A cross-sectional study of SVD-associated ICH; histopathological features

4.1 Introduction

Understanding the prevalence, severity and distribution of SVDs in SVD-associated ICH is important. It helps relate research neuroimaging findings, such as the distribution of MRI SVD biomarkers or amyloid PET tracer uptake, to the underlying histopathology.[95, 252] It may also help inform clinical practice by inferring the likely cause of lobar and non-lobar ICH, which in turn can influence the outcome, such as the risk of recurrent ICH and post-ICH dementia.[59, 105]

Histopathological assessment of brain tissue is the reference standard for assessing the severity and distribution of SVDs associated with ICH. Brain tissue can be sampled in different ways.

During life, a cortical biopsy is the pathological reference standard for diagnosing CAA-associated ICH. However, it is at risk of sampling error. A false negative result can occur because of the patchy distribution of CAA and the small volume of tissue sampled.[27] A false positive result could occur due to the presence of coincidental CAA, which is a common finding in the elderly.[14] There are different histopathological approaches for grading CAA severity and defining CAA-associated ICH. The Vonsattel scale is one of the most widely used scales for grading CAA (Table 4.1). Both Vonsattel grade ≥1 and ≥2 have been used to define a CAA-associated ICH.[164, 253] More recently a consensus rating scale for CAA was developed by Love et al (Table 2.6).[36] Quantifying the diagnostic accuracy of cortical biopsy using these different histopathological approaches is important for guiding clinical management as well as for research in ICH.

After death, brain tissue can be acquired at autopsy. Multiple tissue samples are usually obtained either from one cerebral hemisphere or from the whole brain. Whole brain tissue sampling allows the most detailed assessment of

the severity and distribution of SVDs but is more time consuming and expensive to perform. However, the reliability of sampling one cerebral hemisphere is uncertain given that CAA is reported to have a patchy distribution within cerebral lobes.[27] Therefore, it is important to assess whether the presence and severity of CAA differs between unilateral cerebral hemisphere and whole brain autopsy sampling.

Table 4.1 Vonsattel scale for grading CAA

CAA severity	Features
Grade 0	Absence of amyloid-β staining in vessels
Grade 1	Presence of some patchy amyloid-β staining in an otherwise normal-appearing vessel
Grade 2	Complete replacement of the media by amyloid - wall is thickened
Grade 3	The vessel shows total replacement of the media with amyloid-β and cracking of the vessel wall that creates a "vessel-within-vessel" affecting at least 50% of the circumference of the vessel
Grade 4	Presence of an amyloid-laden vessel with scarring and fibrinoid necrosis

The most advanced degree of CAA present in the specimen in used. CAA = cerebral amyloid angiopathy.

CAA is common in the elderly.[14] Its prevalence increases with age[164] and with the presence of Alzheimer's disease.[14] A large meta-analysis of 24 studies (3520 participants) showed that there is a dose-dependent association of APOE ε4 with histopathologically confirmed CAA.[254] The association between APOE ε4 and CAA remained similar in those with and without dementia, suggesting that it is independent of the known association between APOE ε4 and Alzheimer's disease.[255, 256] There was a non-significantly decreased odds of CAA with APOE ε2 allele possession (OR 0.73, 95% CI 0.53 to 1.00).[254]

Most of the studies assessing the clinical and genetic associations of histopathologically proven CAA were performed in non-ICH general hospital-based autopsy series, autopsy studies of ageing or dementia brain banks, where CAA may have been incidental and asymptomatic. The associations of CAA in symptomatic ICH may be different, and they may also vary according to the type of vessel involved and with the severity of CAA. For example, there is evidence that APOE ε4 is associated with CAA involving the capillaries (CAA-type 1) whereas APOE ε2 is associated with CAA not affecting the capillaries (CAA-type 2).[257] The associations of CAA with age, Alzheimer's disease, hypertension and APOE genotype in symptomatic ICH, accounting for the type of vessel affected (cortical and leptomeningeal arteries and capillaries) and severity of histopathological changes is therefore of interest.

It is often cited that the occipital lobe is the brain region most frequently and severely affected by CAA.[18, 258] This feature has been used to explain a variety of neuroimaging findings in patients with CAA.[208, 252, 259-262] However, the literature on CAA distribution is conflicting, with some studies showing an occipital predominance of CAA, [28, 263, 264] while others do not.[265, 266] The topographical distribution CAA may also vary according to co-existent Alzheimer's pathology.[267] Most of these studies were performed in non-ICH populations or hospital-based autopsy studies, where CAA may be incidental. Little is known about the distribution of CAA in symptomatic ICH, and the effect APOE genotype and Alzheimer's disease have in this patient group.

Participants in the community-based LINCHPIN study were invited to consent to a research brain autopsy in the event of their death. The LINCHPIN brain bank provides systematic and extensively sampled brain autopsy tissue, with standardised assessments for CAA and non-CAA SVD. The histopathology of SVDs in ICH can, therefore, be assessed in detail, using the LINCHPIN brain bank, and these histopathological findings can be related to the clinical characteristics and to neuroimaging features.

4.2 Aims

I aimed to:

- Compare the clinical and diagnostic non-contrast brain CT features of first-ever SVD-associated ICH patients in LATCH consenting to and undergoing LINCHPIN research brain autopsy against those who did not consent, in order to determine selection bias.
- Assess the diagnostic accuracy of CAA assessment in the left cerebral hemisphere against the reference standard of histopathological assessment using systematic whole brain autopsy samples in first-ever SVD-associated ICH.
- Assess the diagnostic accuracy of different histopathological CAA rating approaches in simulated cortical biopsies against the reference standard of histopathological assessment using systematic whole brain autopsy samples in first-ever SVD-associated lobar ICH.
- Evaluate the severity and associations of histopathologically assessed CAA in participants with a first-ever SVD-associated lobar ICH.
- Evaluate the distribution of histopathologically assessed CAA in participants with a first-ever SVD-associated lobar ICH. In particular, to evaluate whether CAA is more frequent and severe in the occipital lobes compared with the rest of the cerebral hemispheres (i.e. occipital predominance of CAA).

4.3 Methods

I used data from the prospective LINCHPIN study (Section 2.1.3.2). I included consecutive adult participants (aged ≥16 years) living in the NHS Lothian Health Board region who had a first-ever SVD-associated ICH between 1st June 2010 and 31st May 2016 inclusive and who underwent a subsequent research brain autopsy.

I excluded patients with exclusively extra-axial intracranial haemorrhage and ICH secondary to an underlying cause other than SVDs. I excluded

LINCHPIN participants with a previous symptomatic ICH to standardise the inception point.

4.3.1 Baseline data collection

The RUSH team collected demographics and the presence of relevant co-morbidities and medication use at the time of ICH by interviewing patients and their relatives and reviewing medical records as described in the methods chapter (Section 2.1.7).

4.3.2 APOE genotyping

APOE genotyping was performed on DNA extracted from peripheral blood or from brain tissue using standard techniques described in Section 2.3. I defined APOE ε2 and APOE ε4 possession if participants had at least one ε2 allele or one ε4 allele respectively.

4.3.3 Research brain autopsy

Research brain autopsy was performed within five days of death according to a standard operating procedure (Section 2.4).[217] SVDs (CAA and non-CAA SVD) and Alzheimer's pathology were assessed by neuropathologists as described in the methods (Section 2.4.1 and 2.4.2).

There is no consensus approach for deriving a global cerebral CAA stage from the Love et al. CAA rating scale.[36] Therefore, to quantify the global cerebral burden of parenchymal and meningeal CAA, I separately summed the parenchymal and meningeal scores for each cerebral lobe and assigned a global parenchymal or meningeal CAA burden category (0=absent, 1-8=mild, 9-16=moderate, and 17-24=severe). I selected these cut-offs based on the distribution of CAA severity ratings in the LINCHPIN brain bank (Figure 4.1).

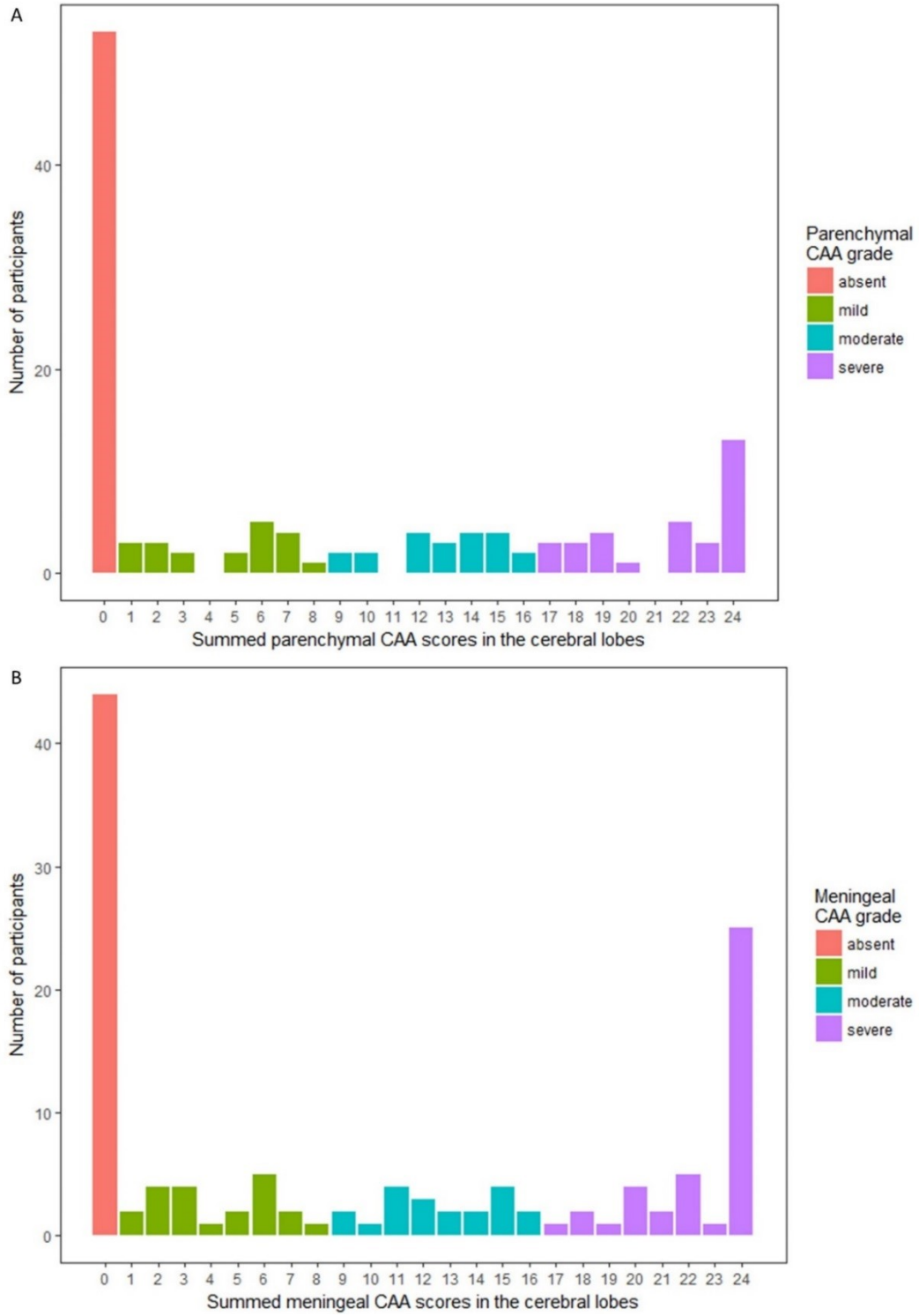
To make the CAA ratings compatible with the non-CAA SVD ratings derived from the left cerebral hemisphere, I separately summed the parenchymal and meningeal scores for each lobe in the left cerebral hemisphere and assigned a left cerebral hemisphere parenchymal or meningeal CAA burden category (0=absent, 1-4=mild, 5-8=moderate, and 9-12=severe, Figure 4.2). I

dichotomised the global cerebral and left cerebral hemisphere parenchymal and meningeal CAA burden categories (absent or mild versus moderate or severe) and the global cerebral and left cerebral hemisphere capillary CAA and vasculopathy ratings (present or absent) for analysis.

For participants with a lobar ICH, I calculated the Vonsattel CAA grade based on the most advanced degree of parenchymal or meningeal CAA present within the cerebral lobe where the ICH was centred (Table 4.1).[164, 253]

Figure 4.1 Distribution of the summed CAA scores across all cerebral lobes in first-ever ICH LINCHPIN participants

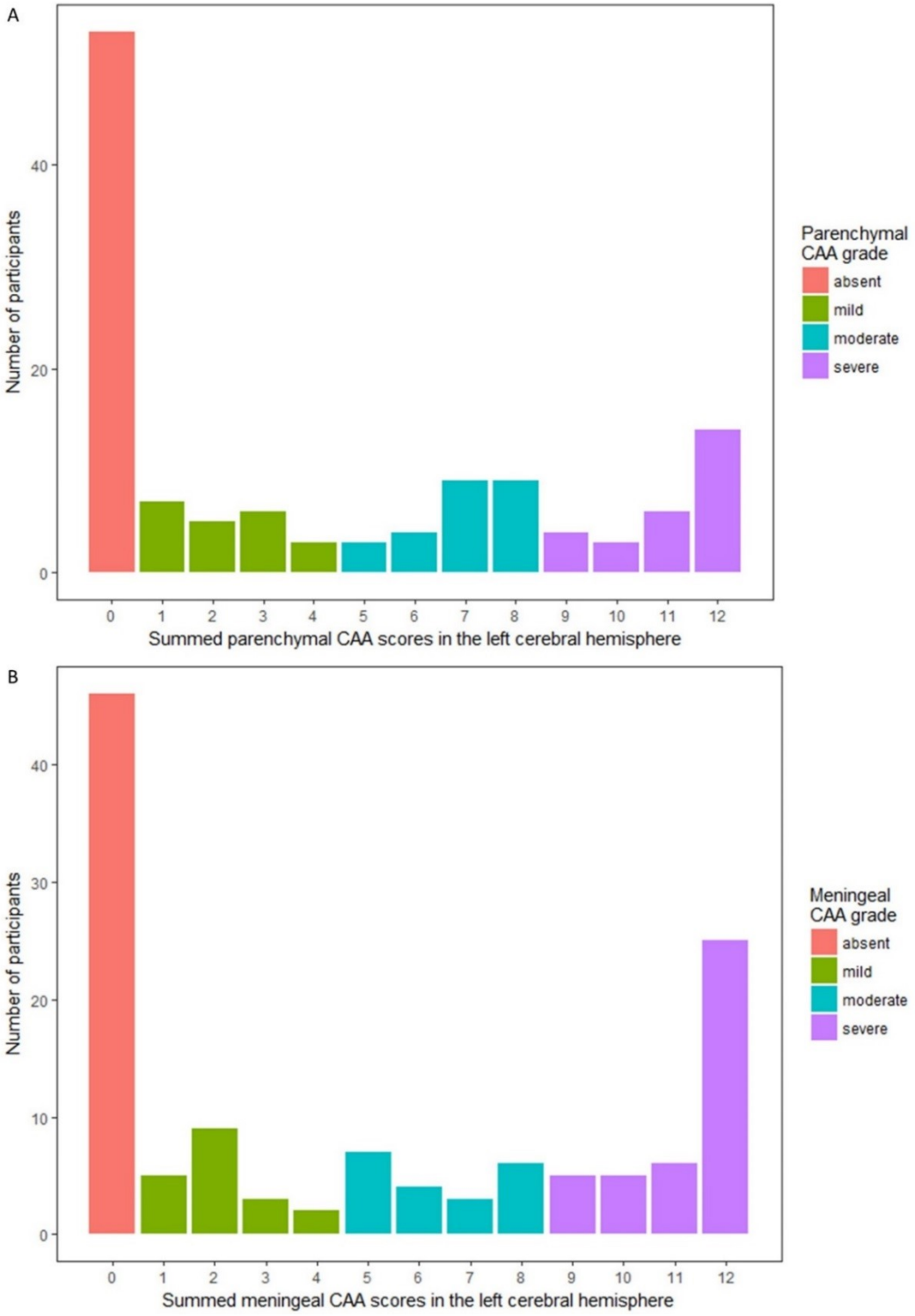
A) Parenchymal CAA. B) Meningeal CAA.



CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome.

Figure 4.2 Distribution of the summed CAA scores in the left cerebral hemisphere in first-ever ICH LINCHPIN participants

A) Parenchymal CAA. B) Meningeal CAA.



CAA = cerebral amyloid angiopathy. ICH – intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome.

4.3.4 Statistical analysis

I used univariable analyses to compare the frequency of baseline clinical characteristics, diagnostic non-contrast brain CT features and APOE genotype between groups using χ^2 test (or Fisher's exact test, where appropriate) for categorical variables and the Mann-Whitney U test for non-normally distributed continuous variables.

I evaluated the diagnostic accuracy of different sampling approaches for histopathological CAA assessment (the left cerebral hemisphere or the cerebral lobe affected by ICH) against the reference standard of global cerebral CAA histopathological assessment using sensitivity and specificity and their 95% CI. I performed pre-specified sensitivity analyses of the diagnostic accuracy of CAA assessment using tissue from the cerebral lobe affected by ICH stratified by age tertiles, given that the specificity of simulated cortical biopsies for CAA was shown to decrease with age.[164]

I used Firth's penalised likelihood logistic regression to assess whether APOE genotype and Thal phase were independently associated with left cerebral hemisphere parenchymal or meningeal CAA and vasculopathy in participants with first-ever lobar ICH because Thal phase showed complete separation between the outcome groups.[268] I performed multivariable logistic regression to assess whether APOE genotype and Thal phase were independently associated with left cerebral hemisphere capillary CAA in participants with first-ever lobar ICH. I pre-specified APOE genotype and Thal phase to include in multivariable models of CAA based on their previous associations with CAA.[254-256] I did not include other variables in these models to reduce overfitting.

To assess the distribution of CAA, I calculated the relative risk (RR) and 95% confidence intervals of moderate or severe parenchymal or meningeal CAA, as well as the presence of capillary CAA or vasculopathy in the occipital lobes compared with the other cerebral lobes.

I performed statistical analyses using R statistical package version 3.4.4., except for the diagnostic accuracy statistics and RR, for which I used VassarStats Clinical Calculator 1.[269]

4.3.5 Missing data

The amount of missing data was low (Table 2.2). APOE genotyping was missing for one participant. I did not impute this missing data point. Instead, I excluded this participant from the relevant analyses.

4.4 Results

4.4.1 Flow of participants

There were 612 patients with spontaneous ICH presumed related to SVDs between 1st June 2010 and 31st May 2016. Three hundred and fifty consented to the LINCHPIN study, including 233 with a first-ever ICH who consented to research brain autopsy (Figure 3.3). One hundred and twenty-six of these participants died and underwent research brain autopsy, including 71 with a lobar ICH and 55 with a non-lobar ICH. The median time between ICH onset and research brain autopsy was 12 days (IQR 6-162 days, range 1-2405 days).

4.4.2 Comparison of first-ever ICH participants who had a research brain autopsy versus those who did not

To assess for selection bias in the LINCHPIN brain bank, I compared the clinical and baseline imaging features between LINCHPIN participants recruited during the community-based LATCH cohort study (1st June 2010 until 31st May 2013 inclusive) who consented to and underwent research brain autopsy against LATCH patients who did not consent to research brain autopsy.

4.4.2.1 Comparison of LATCH patients with first-ever SVD-associated ICH who did and did not consent to research brain autopsy

During the community-based LATCH cohort study, 128 first-ever SVD-associated ICH patients consented to research brain autopsy as part of LINCHPIN (Figure 3.4). Consenters were generally similar to the rest of the LATCH cohort, except that they were significantly less likely to have had a previous ischaemic stroke or myocardial infarction (Table 4.2).

The frequencies of lobar ICH location and subarachnoid haemorrhage on the diagnostic brain CT were higher in those who consented to research brain autopsy compared with the rest of the LATCH cohort, while the proportion with infratentorial ICH was lower in research brain autopsy consenters (Table 4.3).

Table 4.2 Baseline clinical features in first-ever SVD-associated ICH LATCH patients who consented to research brain autopsy versus those who did not

	Research autopsy consent (n=128)		No research autopsy consent (n=291)		p value
Age (years); median (IQR)	77	(70-85)	77	(66-83)	0.366
Sex					
Female	66	(52)	159	(55)	0.561
Male	62	(48)	132	(45)	
Co-morbidities*					
Hypertension	76	(59)	196	(68)	0.105
Ischaemic stroke	14	(11)	55	(19)	0.042
Transient ischaemic attack	14	(11)	31	(11)	0.940
Dementia	16	(13)	36	(12)	0.971
Diabetes	12	(9)	35	(12)	0.422
Atrial fibrillation	23	(18)	67	(23)	0.239
Myocardial infarction	5	(4)	32	(11)	0.018
Hyperlipidaemia	20	(16)	55	(19)	0.412
Smoking status					
Current	24	(19)	68	(24)	0.131
Ex-smoker	40	(31)	107	(37)	
Never	64	(50)	114	(39)	
Pre-ICH modified Rankin scale					
0	56	(44)	83	(29)	
1	16	(13)	67	(23)	
2	23	(18)	69	(24)	
3	25	(20)	57	(20)	
4	5	(4)	10	(4)	
5	2	(2)	1	(0)	
Pre-ICH modified Rankin scale; median (IQR)	2	(1-4)	2	(1-3)	0.134
Medications on admission					
Antiplatelet drug(s)	55	(43)	122	(42)	0.842
Anticoagulant drug(s)	13	(10)	43	(15)	0.200
Antihypertensive drug(s)	56	(44)	146	(50)	0.226
Admission GCS score; median (IQR) †	14	(10-15)	14	(10-15)	0.129

Data are n (%) or median (IQR). * missing for 1 patient. † missing for4 patients. GCS = Glasgow coma scale. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. SVD = small vessel disease.

Table 4.3 ICH location and baseline imaging features between first-ever SVD-associated ICH LATCH patients who consented to research brain autopsy versus those who did not

	Research autopsy consent (n=128)	**No research autopsy consent (n=291)**	**p value**
ICH location			
Lobar	70 (55)	138 (47)	
Deep	50 (39)	109 (38)	0.036
Infratentorial	8 (6)	44 (15)	
ICH volume; median (IQR) ‡	18 (6-49)	20 (5-55)	0.998
Intraventricular haemorrhage	52 (41)	139 (48)	0.176
Subarachnoid haemorrhage	64 (50)	115 (40)	0.046
Subdural haemorrhage	10 (8)	32 (11)	0.317
CT SVD score^			
0	39 (32)	98 (35)	
1	49 (40)	106 (38)	0.929
2	27 (22)	64 (23)	
3	7 (6)	14 (5)	

Data are n (%) or median (IQR). ‡ missing for 5 patients diagnosed by autopsy. ^ missing for 15 patients without a diagnostic CT. CT = computed tomography. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. SVD = small vessel disease.

4.4.2.2 Comparison of LATCH patient with first-ever SVD-associated ICH who did and did not undergo research brain autopsy

Seventy-two LATCH patients with first-ever ICH died and underwent research brain autopsy (Figure 3.4). Donors were significantly older, more likely to have pre-ICH dementia and worse pre-ICH levels of functioning compared with the rest of the LATCH cohort (Table 4.4). They also had larger ICHs with more frequent subarachnoid haemorrhage (Table 4.5).

Table 4.4 Baseline clinical features in first-ever SVD-associated ICH LATCH patients who underwent research brain autopsy versus those who did not

	Research autopsy obtained (n=72)		No research autopsy obtained (n=347)		p value
Age (years); median (IQR)	81	(76-86)	76	(66-83)	<0.001
Sex					
Female	40	(56)	185	(53)	0.729
Male	32	(44)	162	(47)	
Co-morbidities*					
Hypertension	45	(63)	227	(66)	0.615
Ischaemic stroke	10	(14)	59	(17)	0.511
Transient ischaemic attack	8	(11)	37	(11)	0.917
Dementia	14	(19)	38	(11)	0.047
Diabetes	7	(10)	40	(12)	0.653
Atrial fibrillation	19	(26)	71	(21)	0.270
Myocardial infarction	5	(7)	32	(9)	0.531
Hyperlipidaemia	11	(15)	64	(19)	0.517
Smoking status					
Current	13	(18)	79	(23)	0.256
Ex-smoker	22	(31)	125	(36)	
Never	37	(51)	141	(41)	
Pre-morbid modified Rankin scale					
0	17	(24)	122	(36)	
1	13	(18)	70	(20)	
2	15	(21)	77	(22)	
3	19	(27)	63	(18)	
4	5	(7)	10	(3)	
5	2	(3)	1	(0)	
Pre-morbid modified Rankin scale; median (IQR)	3	(2-4)	2	(1-3)	0.006
Medications on admission					
Antiplatelet drug(s)	36	(50)	141	(41)	0.143
Anticoagulant drug(s)	10	(14)	46	(13)	0.886
Antihypertensive drug(s)	34	(47)	168	(48)	0.854
Admission GCS score; median (IQR) †	13	(10-14)	14	(10-15)	0.128

Data are n (%), median (IQR) or mean (SD). * missing for 1 patient. † missing for4 patients. GCS = Glasgow coma scale. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. SVD = small vessel disease.

Table 4.5 ICH and baseline imaging features in first-ever SVD-associated ICH LATCH patients who underwent research brain autopsy versus those who did not

	Research autopsy obtained (n=72)		No research autopsy obtained (n=347)		p value
ICH location					
Lobar	40	(56)	168	(48)	0.258
Deep	27	(38)	132	(38)	
Infratentorial	5	(7)	47	(14)	
ICH volume; median (IQR) ‡	29	(12-68)	18	(5-52)	0.008
Intraventricular haemorrhage	37	(51)	154	(44)	0.277
Subarachnoid haemorrhage	40	(56)	139	(40)	0.016
Subdural haemorrhage	8	(11)	34	(10)	0.736
CT SVD score^					
0	20	(28)	117	(35)	0.628
1	30	(42)	125	(38)	
2	16	(23)	75	(23)	
3	5	(7)	16	(5)	

Data are n (%), median (IQR) or mean (SD). ‡ missing for 5 patients diagnosed by autopsy. ^ missing for 15 patients without a diagnostic CT. CT = computed tomography. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. SVD = small vessel disease.

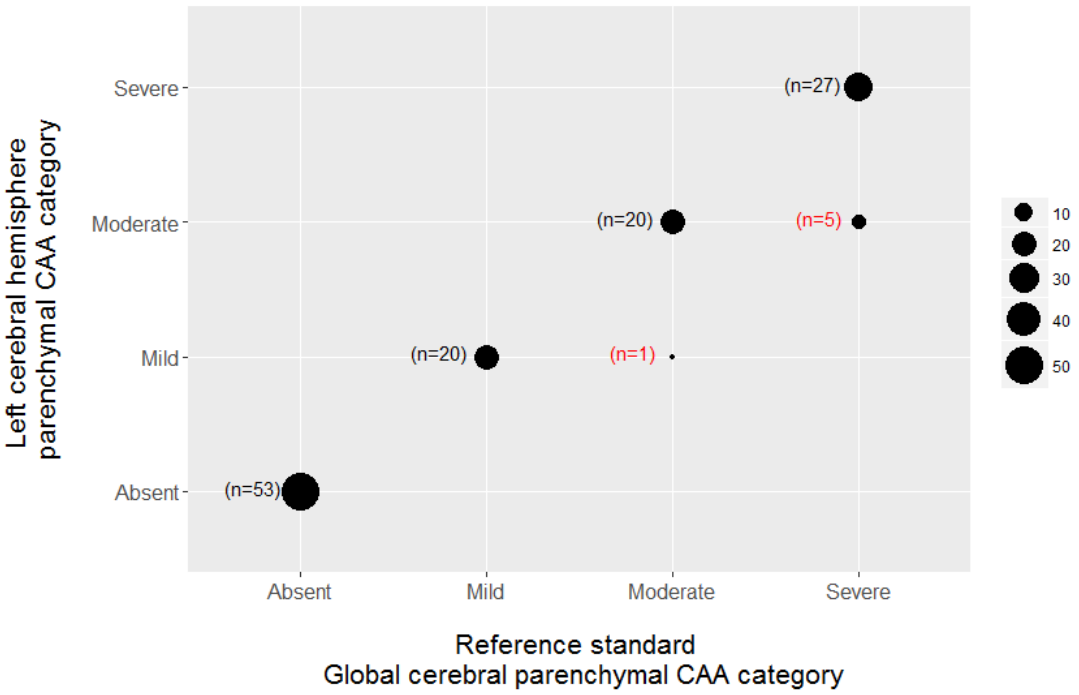
4.4.3 Histopathological assessment of CAA and vasculopathy in the left cerebral hemisphere versus systematic whole brain autopsy in first-ever SVD-associated ICH

To determine whether the histopathological assessment of CAA in the left cerebral hemisphere is representative of total brain CAA, I compared the severity of CAA and vasculopathy in the left cerebral hemisphere against the reference standard of global cerebral CAA histopathological assessment in all 126 LINCHPIN brain bank participants with first-ever SVD-associated ICH, regardless of ICH location.

4.4.3.1 Parenchymal CAA

Figure 4.3 shows the parenchymal CAA severity in the left cerebral hemisphere compared with the global cerebral parenchymal CAA severity (reference standard). Seventy-three participants had absent or mild global cerebral parenchymal CAA, all of whom had absent or mild left cerebral hemisphere parenchymal CAA, resulting in a specificity of 100% (95% CI 94-100). Fifty-three participants had moderate or severe global cerebral parenchymal CAA, 52 of whom had moderate or severe left cerebral hemisphere parenchymal CAA, giving a sensitivity of 98% (95 %CI 89-100).

Figure 4.3 Bubble plot of left cerebral hemisphere parenchymal CAA severity against global cerebral parenchymal CAA severity in first-ever ICH participants.



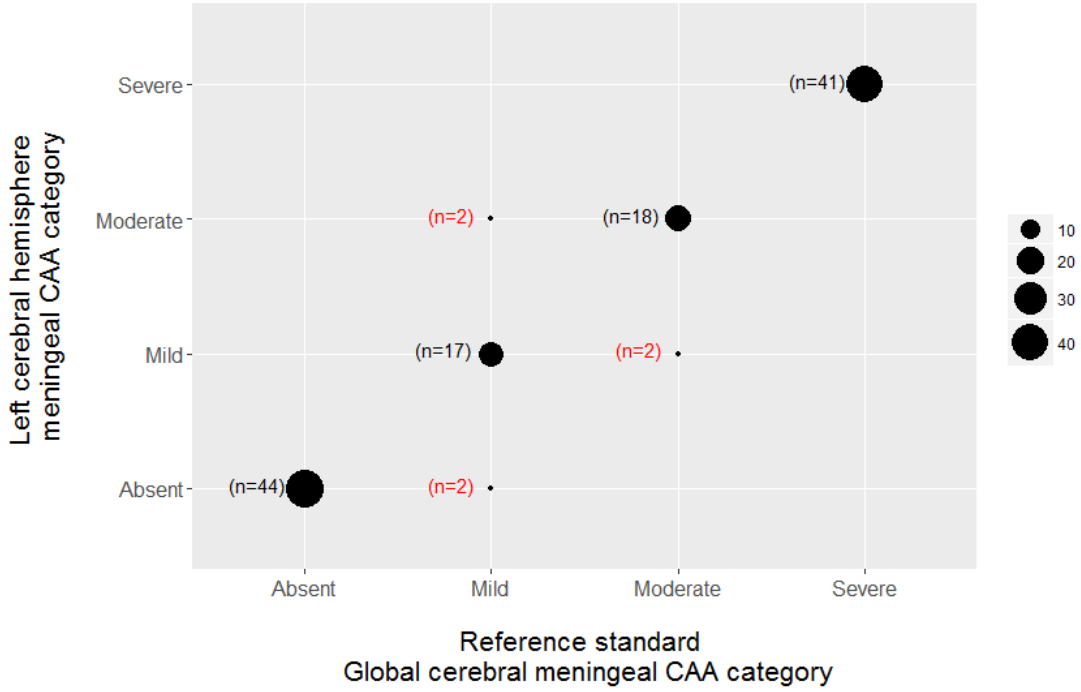
CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

4.4.3.2 Meningeal CAA

The meningeal CAA severity in the left cerebral hemisphere compared with the global cerebral meningeal CAA severity (reference standard) is shown in

Figure 4.4. Sixty-five participants had absent or mild global cerebral meningeal CAA, 63 of whom had absent or mild left cerebral hemisphere meningeal CAA, resulting in a specificity of 97% (95% CI 88-99). Sixty-one participants had moderate or severe global meningeal parenchymal CAA, 59 of whom had moderate or severe left cerebral hemisphere meningeal CAA, resulting in a sensitivity of 97% (95% CI 88-99).

Figure 4.4 Bubble plot of left cerebral hemisphere meningeal CAA severity against global cerebral meningeal CAA severity in first-ever ICH participants.



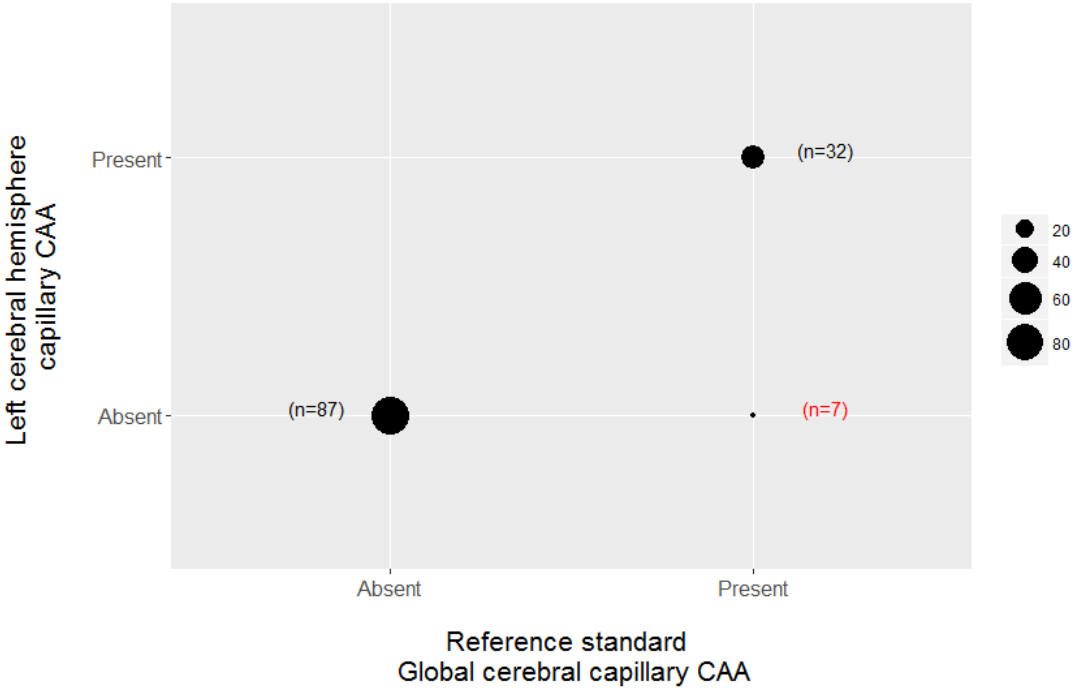
CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

4.4.3.3 Capillary CAA

The presence or absence of capillary CAA in the left cerebral hemisphere compared with both cerebral hemispheres (reference standard) is shown in Figure 4.5. Eighty-seven participants had no capillary CAA on global cerebral assessment, all of whom had no capillary CAA in the left cerebral hemisphere, resulting in a specificity of 100% (95% CI 95-100). Thirty-nine

participants had capillary CAA present on global cerebral assessment, 32 of whom had capillary CAA present in the left cerebral hemisphere, resulting in a sensitivity of 82% (95% CI 66-92).

Figure 4.5 Bubble plot of left cerebral hemisphere capillary CAA against global cerebral capillary CAA in first-ever ICH participants.

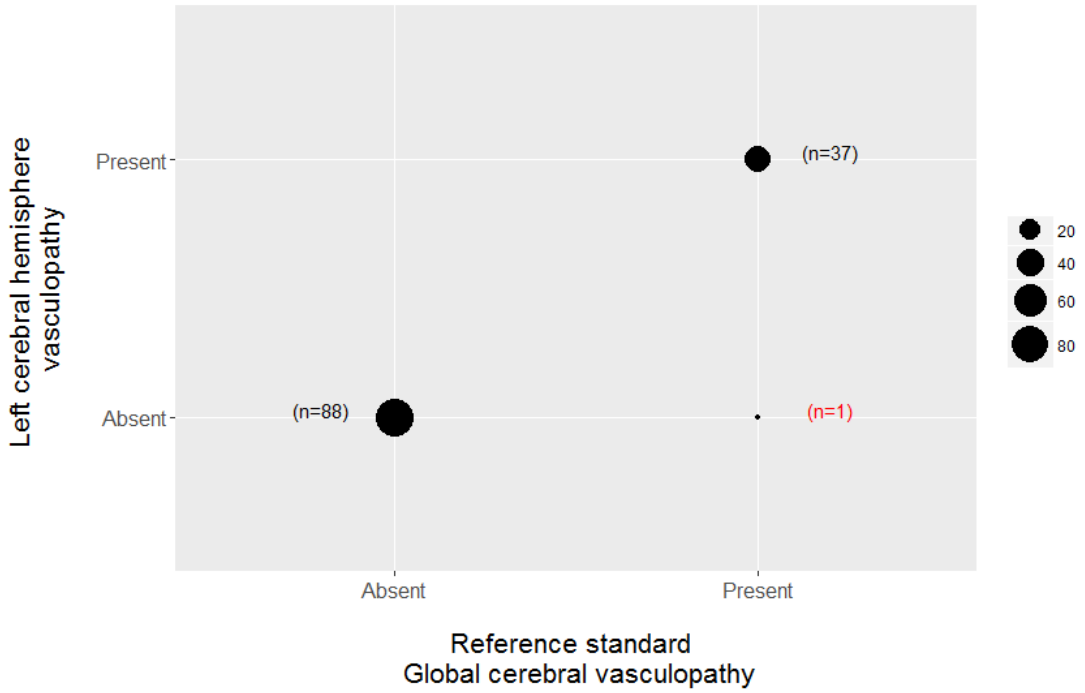


CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

4.4.3.4 Vasculopathy

The presence or absence of vasculopathy in the left cerebral hemisphere compared with both cerebral hemispheres (reference standard) is shown in Figure 4.6. Eighty-eight participants had no vasculopathy on global cerebral assessment, all of whom had no vasculopathy in the left cerebral hemisphere, resulting in a specificity of 100% (95% CI 95-100). Thirty-eight participants had vasculopathy present on global cerebral assessment, 37 of whom had vasculopathy in the left cerebral hemisphere, resulting in a sensitivity of 97% (95% CI 85-100).

Figure 4.6 Bubble plot of left cerebral hemisphere vasculopathy against global cerebral vasculopathy in first-ever ICH participants.



ICH = intracerebral haemorrhage.

4.4.4 Histopathological assessment of CAA and vasculopathy in the cerebral lobe affected by ICH versus systematic whole brain autopsy in first-ever lobar ICH

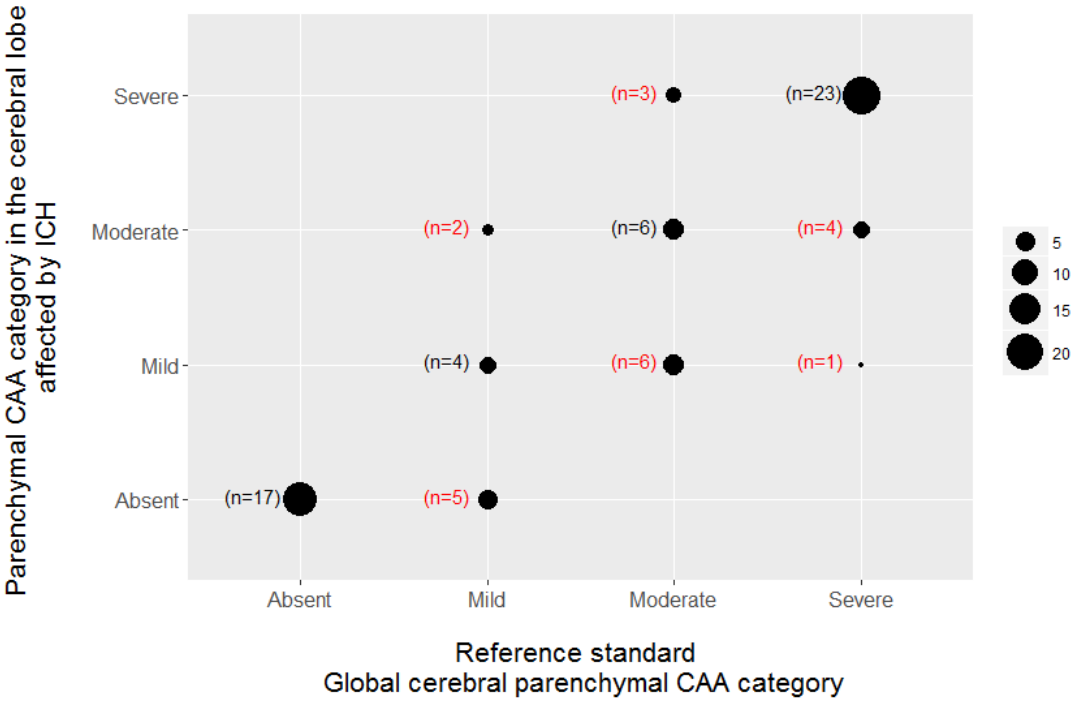
To simulate a cortical biopsy, I assessed the diagnostic accuracy of histopathological assessment of CAA and vasculopathy in the cerebral lobe affected by ICH using the Love et al[36] and Vonsattel[253] scales against the reference standard of global cerebral CAA histopathological assessment using the Love et al scale[36].

Seventy-one LINCHPIN participants who underwent a research autopsy had a first-ever SVD-associated lobar ICH. The ICH epicentre was located in the frontal lobe in 34 (48%), parietal lobe in 16 (23%), temporal lobe in 12 (17%) and the occipital lobe in 9 (13%).

4.4.4.1 Parenchymal CAA

The parenchymal CAA severity in the cerebral lobe where the ICH epicentre was located compared with the global cerebral parenchymal CAA severity (reference standard) is shown in Figure 4.7. Twenty-eight participants had absent or mild global cerebral parenchymal CAA, 26 of whom had absent or mild parenchymal CAA in the cerebral lobe where the ICH epicentre was located, resulting in a specificity of 93% (95%CI 75-99). Forty-three participants had moderate or severe global cerebral parenchymal CAA, 36 of whom had moderate or severe parenchymal CAA in the cerebral lobe where the ICH epicentre was located, resulting in a sensitivity of 84% (95% CI 69-93).

Figure 4.7 Bubble plot of parenchymal CAA severity in the lobe containing the ICH epicentre against global cerebral parenchymal CAA severity in first-ever lobar ICH participants.



CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

I performed a sensitivity analysis of the diagnostic accuracy of parenchymal CAA stratified by age at the time of the index ICH (Table 4.6 and Table 4.7).

Table 4.6 Cross-tabulations of parenchymal CAA severity in the lobe containing the ICH epicentre against the global cerebral parenchymal CAA severity, stratified by age at the time of the index ICH

Age at time of index ICH 56 to 79 years			
Cerebral lobe affected by ICH parenchymal CAA (Index test)	**Global cerebral parenchymal CAA (Reference standard)**		
	Absent/mild	Moderate/severe	Total
Absent/mild	10	3	13
Moderate/severe	0	12	12
Total	10	15	25
Age at time of index ICH 80 to 84 years			
Cerebral lobe affected by ICH parenchymal CAA (Index test)	**Global cerebral parenchymal CAA (Reference standard)**		
	Absent/mild	Moderate/severe	Total
Absent/mild	6	1	7
Moderate/severe	0	15	15
Total	6	16	22
Age at time of index ICH 85 to 95 years			
Cerebral lobe affected by ICH parenchymal CAA (Index test)	**Global cerebral parenchymal CAA (Reference standard)**		
	Absent/mild	Moderate/severe	Total
Absent/mild	10	3	13
Moderate/severe	2	9	11
Total	12	12	24

Data are number. CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage

Table 4.7 Diagnostic test accuracy of moderate/severe parenchymal CAA severity in the lobe containing the ICH epicentre using moderate/severe global parenchymal CAA severity as the reference standard cut off, stratified by age at the time of the index ICH

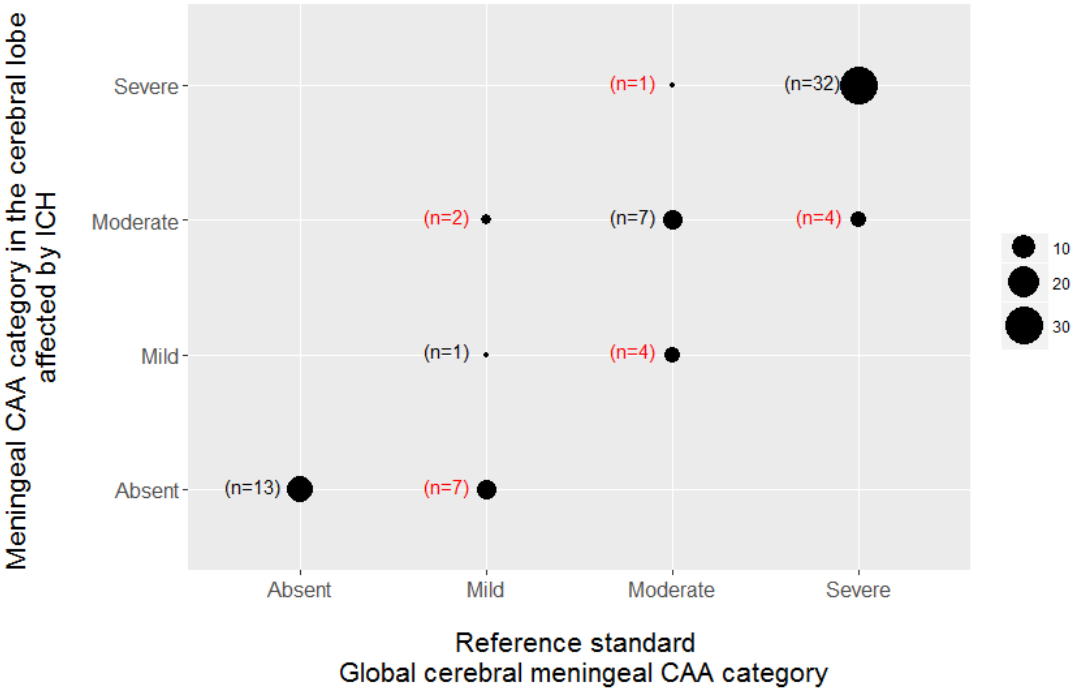
	Age at time of index ICH 56 to 79 years	**Age at time of index ICH 80 to 84 years**	**Age at time of index ICH 85 to 95 years**
Sensitivity	80 (51-95)	94 (68-100)	75 (43-93)
Specificity	100 (66-100)	100 (52-100)	83 (51-97)

Data are percentage (95% CI). CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

4.4.4.2 Meningeal CAA

The meningeal CAA severity in the cerebral lobe where the ICH epicentre was located compared with the global cerebral meningeal CAA severity (reference standard) is shown in Figure 4.8. Twenty-three participants had absent or mild global meningeal parenchymal CAA, 21 of whom had absent or mild meningeal CAA in the cerebral lobe where the ICH epicentre was located, resulting in a specificity of 91% (95% CI 70-98). Forty-eight participants had moderate or severe global cerebral meningeal CAA, 44 of whom had moderate or severe meningeal CAA in the cerebral lobe where the ICH epicentre was located, resulting in a sensitivity of 92% (95% CI 79-97).

Figure 4.8 Bubble plot of meningeal CAA severity in the lobe containing the ICH epicentre against global cerebral meningeal CAA severity in first-ever lobar ICH participants.



CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

I performed a sensitivity analysis of the diagnostic accuracy of meningeal CAA stratified by age at the time of the index ICH (Table 4.8 and Table 4.9).

Table 4.8 Cross-tabulations of meningeal CAA severity in the lobe containing the ICH epicentre against the global cerebral meningeal CAA severity, stratified by age at the time of the index ICH

Age at time of index ICH 56 to 79 years			
Cerebral lobe affected by ICH meningeal CAA (Index test)	**Global cerebral meningeal CAA (Reference standard)**		
	Absent/mild	Moderate/severe	Total
Absent/mild	9	1	10
Moderate/severe	1	14	15
Total	10	15	25
Age at time of index ICH 80 to 84 years			
Cerebral lobe affected by ICH meningeal CAA (Index test)	**Global cerebral meningeal CAA (Reference standard)**		
	Absent/mild	Moderate/severe	Total
Absent/mild	5	2	7
Moderate/severe	1	14	15
Total	6	16	22
Age at time of index ICH 85 to 95 years			
Cerebral lobe affected by ICH meningeal CAA (Index test)	**Global cerebral meningeal CAA (Reference standard)**		
	Absent/mild	Moderate/severe	Total
Absent/mild	7	1	8
Moderate/severe	0	16	16
Total	7	17	24

Data are number. CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage

Table 4.9 Diagnostic test accuracy of moderate/severe meningeal CAA severity in the lobe containing the ICH epicentre using moderate/severe global meningeal CAA severity as the reference standard cut off, stratified by age at the time of the index ICH

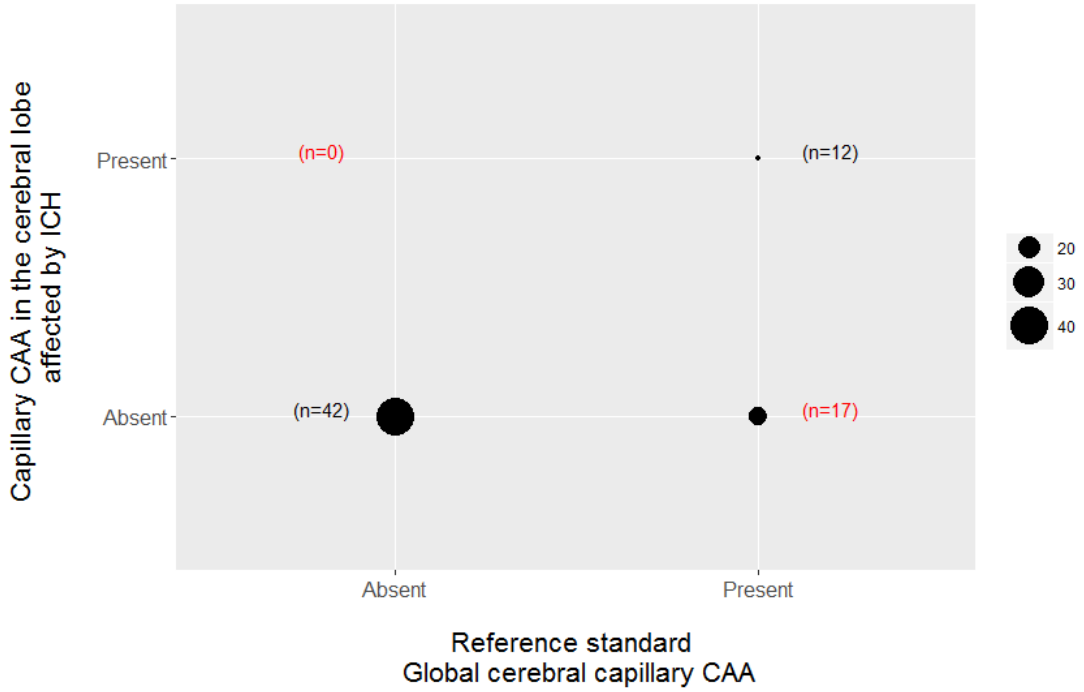
	Age at time of index ICH 56 to 79 years	**Age at time of index ICH 80 to 84 years**	**Age at time of index ICH 85 to 95 years**
Sensitivity	93 (66-100)	88 (60-98)	94 (69-100)
Specificity	90 (54-99)	83 (36-99)	100 (56-100)

Data are percentage (95% CI). CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

4.4.4.3 Capillary CAA

The presence or absence of capillary CAA in the cerebral lobe where the ICH epicentre was located compared with both cerebral hemispheres (reference standard) is shown in Figure 4.9. Forty-two participants had no capillary CAA on global cerebral assessment, all of whom had no capillary CAA in the cerebral lobe where the ICH epicentre was located, resulting in a specificity of 100% (95% CI 90-100). Twenty-nine participants had capillary CAA present on global cerebral assessment, 12 of whom had capillary CAA present in the cerebral lobe where the ICH epicentre was located, resulting in a sensitivity of 41% (95% CI 24-61).

Figure 4.9 Bubble plot of capillary CAA in the lobe containing the ICH epicentre against global cerebral capillary CAA in first-ever lobar ICH participants.



CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

I performed a sensitivity analysis of the diagnostic accuracy of capillary CAA stratified by age at the time of the index ICH (Table 4.10 and Table 4.11).

Table 4.10 Cross-tabulations of capillary CAA in the lobe containing the ICH epicentre against the global cerebral capillary CAA, stratified by age at the time of the index ICH

Age at time of index ICH 56 to 79 years			
Cerebral lobe affected by ICH capillary CAA (Index test)	**Global cerebral capillary CAA (Reference standard)**		
	Absent	Present	Total
Absent	15	7	22
Present	0	3	3
Total	15	10	25
Age at time of index ICH 80 to 84 years			
Cerebral lobe affected by ICH capillary CAA (Index test)	**Global cerebral capillary CAA (Reference standard)**		
	Absent	Present	Total
Absent	12	5	17
Present	0	5	5
Total	12	10	22
Age at time of index ICH 85 to 95 years			
Cerebral lobe affected by ICH capillary CAA (Index test)	**Global cerebral capillary CAA (Reference standard)**		
	Absent	Present	Total
Absent	15	5	20
Present	0	4	4
Total	15	9	24

Data are number. CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

Table 4.11 Diagnostic test accuracy of capillary CAA presence in the lobe containing the ICH epicentre using global cerebral capillary CAA presence as the reference standard cut off, stratified by age at the time of the index ICH

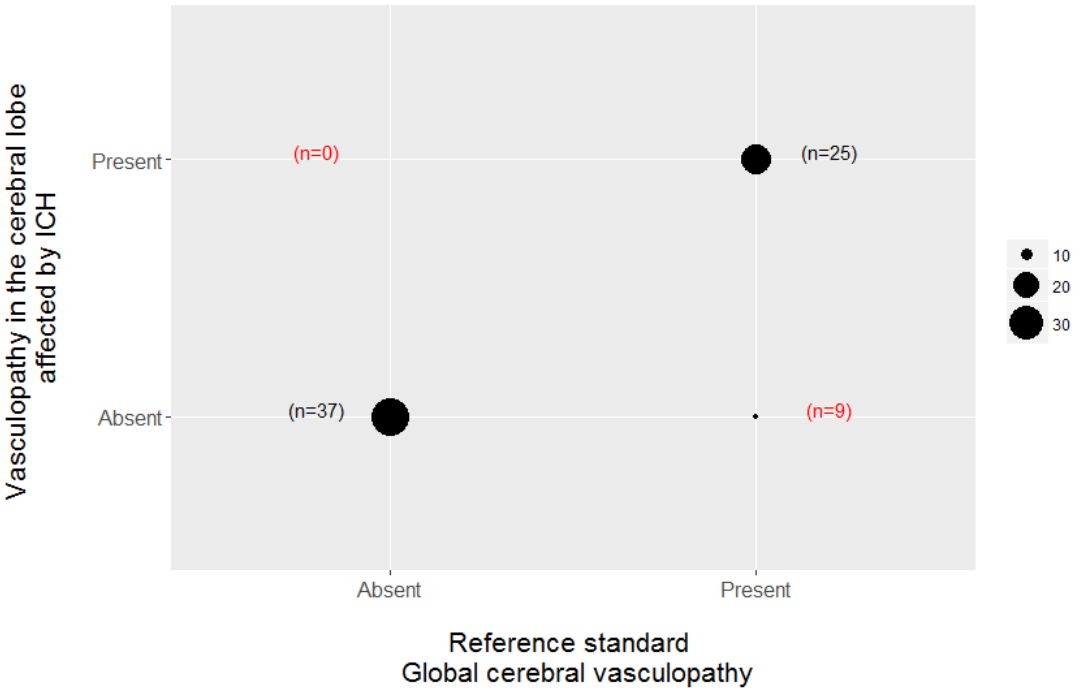
	Age at time of index ICH 56 to 79 years	**Age at time of index ICH 80 to 84 years**	**Age at time of index ICH 85 to 95 years**
Sensitivity	30 (8-65)	50 (20-80)	44 (15-77)
Specificity	100 (75-100)	100 (70-100)	100 (75-100)

Data are percentage (95% CI). CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

4.4.4.4 Vasculopathy

The presence or absence of vasculopathy in the cerebral lobe where the ICH epicentre was located compared with both cerebral hemispheres (reference standard) is shown in Figure 4.10. Thirty-seven participants had no vasculopathy on global assessment, all of whom had no vasculopathy in the cerebral lobe where the ICH epicentre was located, resulting in a specificity of 100% (95% CI 88-100). Thirty-four participants had vasculopathy present on global cerebral assessment, 25 of whom had vasculopathy in the cerebral lobe where the ICH epicentre was located, resulting in a sensitivity of 74% (95% CI 55-86).

Figure 4.10 Bubble plot of vasculopathy in the lobe containing the ICH epicentre against global cerebral vasculopathy in first-ever lobar ICH participants.



ICH = intracerebral haemorrhage.

I performed a sensitivity analysis of the diagnostic accuracy of vasculopathy stratified by age at the time of the index ICH (Table 4.12 and Table 4.13).

Table 4.12 Cross-tabulations of vasculopathy in the lobe containing the ICH epicentre against the global cerebral vasculopathy, stratified by age at the time of the index ICH

Age at time of index ICH 56 to 79 years			
Cerebral lobe affected by ICH vasculopathy (Index test)	**Global cerebral vasculopathy (Reference standard)**		
	Absent	Present	Total
Absent	11	4	15
Present	0	10	10
Total	11	14	25
Age at time of index ICH 80 to 84 years			
Cerebral lobe affected by ICH vasculopathy (Index test)	**Global cerebral vasculopathy (Reference standard)**		
	Absent	Present	Total
Absent	12	1	13
Present	0	9	9
Total	12	10	22
Age at time of index ICH 85 to 95 years			
Cerebral lobe affected by ICH vasculopathy (Index test)	**Global cerebral vasculopathy (Reference standard)**		
	Absent	Present	Total
Absent	14	4	18
Present	0	6	6
Total	14	10	24

Data are number. CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

Table 4.13 Diagnostic test accuracy of the presence of vasculopathy in the lobe containing the ICH epicentre using global cerebral vasculopathy as the reference standard cut off, stratified by age at the time of the index ICH

	Age at time of index ICH 56 to 79 years	**Age at time of index ICH 80 to 84 years**	**Age at time of index ICH 85 to 95 years**
Sensitivity	71 (42-90)	90 (54-99)	60 (27-86)
Specificity	100 (68-100)	100 (70-100)	100 (73-100)

Data are percentage (95% CI). CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

4.4.4.5 Presence of any CAA (Vonsattel grade ≥1)

The presence of any CAA in the cerebral lobe where the ICH epicentre was located (Vonsattel grade ≥1) compared with the global cerebral parenchymal CAA severity (reference standard) is shown in Figure 4.11. Twenty-eight participants had absent or mild global cerebral parenchymal CAA, 20 of whom had Vonsattel grade 0 in the cerebral lobe where the ICH epicentre was located, resulting in a specificity of 71% (95% CI 51-86). Forty-three participants had moderate or severe global cerebral parenchymal CAA, all of whom had Vonsattel grade ≥1 in the cerebral lobe where the ICH epicentre was located, resulting in a sensitivity of 100% (95% CI 90-100).

Figure 4.11 Bubble plot of Vonsattel grade ≥1 in the lobe containing the ICH epicentre against global cerebral parenchymal CAA in first-ever lobar ICH participants.

CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

I performed a sensitivity analysis of the diagnostic accuracy of Vonsattel grade ≥1 stratified by age at the time of the index ICH (Table 4.14 and 4.15).

Table 4.14 Cross-tabulations of the Vonsattel grade of CAA in the lobe containing the ICH epicentre against the global cerebral parenchymal CAA severity, stratified by age at the time of the index ICH

Age at time of index ICH 56 to 79 years			
Cerebral lobe affected by ICH (Index test)	**Global cerebral parenchymal CAA (Reference standard)**		
	Absent/mild	Moderate/severe	Total
Vonsattel grade 0	9	0	9
Vonsattel grade ≥1	1	15	16
Total	10	15	25
Age at time of index ICH 80 to 84 years			
Cerebral lobe affected by ICH (Index test)	**Global cerebral parenchymal CAA (Reference standard)**		
	Absent/mild	Moderate/severe	Total
Vonsattel grade 0	5	0	5
Vonsattel grade ≥1	1	16	17
Total	6	16	22
Age at time of index ICH 85 to 95 years			
Cerebral lobe affected by ICH (Index test)	**Global cerebral parenchymal CAA (Reference standard)**		
	Absent/mild	Moderate/severe	Total
Vonsattel grade 0	6	0	6
Vonsattel grade ≥1	6	12	18
Total	12	12	24

Data are number. CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

Table 4.15 Diagnostic test accuracy of Vonsattel grade ≥1 in the lobe containing the ICH epicentre using moderate/severe global parenchymal CAA severity as the reference standard cut off, stratified by age at the time of the index ICH

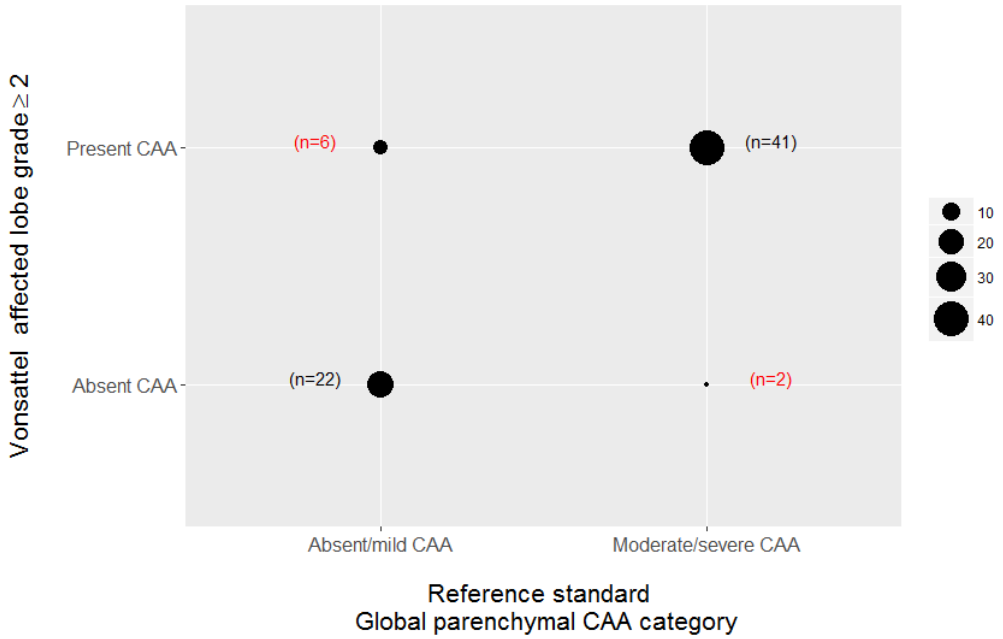
	Age at time of index ICH 56 to 79 years		**Age at time of index ICH 80 to 84 years**		**Age at time of index ICH 85 to 95 years**	
Sensitivity	100	(75-100)	100	(76-100)	100	(70-100)
Specificity	90	(54-99)	83	(36-99)	50	(22-78)

Data are percentage (95% CI). CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

4.4.4.6 Presence of complete replacement of a vessel wall with amyloid-β (Vonsattel grade ≥2)

The presence of complete replacement of a vessel wall with β-amyloid in the cerebral lobe where the ICH epicentre was located (Vonsattel grade ≥2) compared with the global cerebral parenchymal CAA severity (reference standard) is shown in Figure 4.12. Twenty-eight participants had absent or mild global cerebral parenchymal CAA, 22 of whom had Vonsattel grade <2 in the cerebral lobe where the ICH epicentre was located, resulting in a specificity of 79% (95% CI 59-91). Forty-three participants had moderate or severe global cerebral parenchymal CAA, 41 of whom had Vonsattel grade ≥2 in the cerebral lobe where the ICH epicentre was located, resulting in a sensitivity of 95% (95% CI 83-99).

Figure 4.12 Bubble plot of Vonsattel grade ≥2 in the lobe containing the ICH epicentre against global cerebral parenchymal CAA in first-ever lobar ICH participants.



CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

I performed a sensitivity analysis of the diagnostic accuracy of Vonsattel grade ≥2 stratified by age at the time of the index ICH (Table 4.16 and 4.17).

Table 4.16 Cross-tabulations of the Vonsattel grade of CAA in the lobe containing the ICH epicentre against the global cerebral parenchymal CAA severity, stratified by age at the time of the index ICH

Age at time of index ICH 56 to 79 years			
Cerebral lobe affected by ICH (Index test)	**Global cerebral parenchymal CAA (Reference standard)**		
	Absent/mild	Moderate/severe	Total
Vonsattel grade <2	9	1	10
Vonsattel grade ≥2	1	14	15
Total	10	15	25
Age at time of index ICH 80 to 84 years			
Cerebral lobe affected by ICH (Index test)	**Global cerebral parenchymal CAA (Reference standard)**		
	Absent/mild	Moderate/severe	Total
Vonsattel grade <2	5	1	6
Vonsattel grade ≥2	1	15	16
Total	6	16	22
Age at time of index ICH 85 to 95 years			
Cerebral lobe affected by ICH (Index test)	**Global cerebral parenchymal CAA (Reference standard)**		
	Absent/mild	Moderate/severe	Total
Vonsattel grade <2	8	0	8
Vonsattel grade ≥2	4	12	16
Total	12	12	24

Data are number. CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

Table 4.17 Diagnostic test accuracy of Vonsattel grade ≥2 CAA severity in the lobe containing the ICH epicentre using moderate/severe global parenchymal CAA severity as the reference standard cut off, stratified by age at the time of the index ICH

	Age at time of index ICH 56 to 79 years		**Age at time of index ICH 80 to 84 years**		**Age at time of index ICH 85 to 95 years**	
Sensitivity	93	(66-100)	94	(68-100)	100	(70-100)
Specificity	90	(54-99)	83	(36-99)	67	(35-89)

Data are percentage (95% CI). CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

4.4.5 Pathological severity and associations of SVDs in first-ever SVD-associated ICH

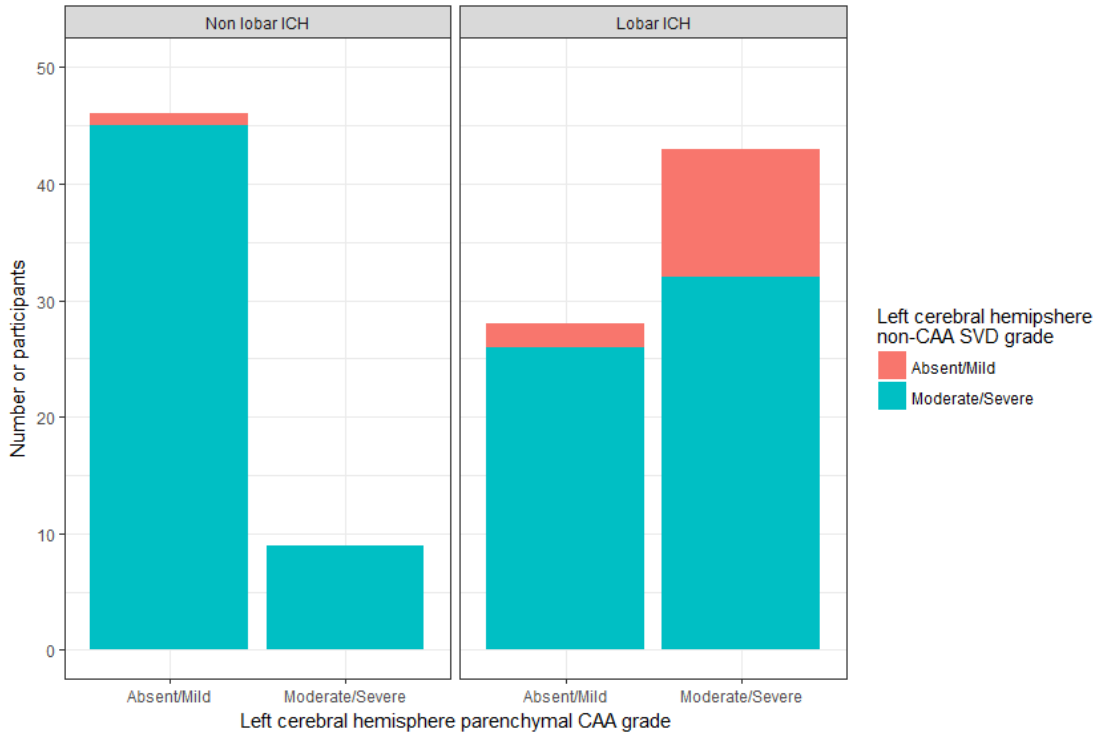
In this section, I have used the histopathology ratings from the left cerebral hemisphere to assess the severity of SVDs in first-ever SVD-associated ICH for two reasons. Firstly, I wanted to make the CAA ratings similar to the non-CAA SVD ratings, which were only performed in the left cerebral hemisphere. Secondly, I have shown in section 4.4.3 that the presence and severity of CAA and vasculopathy in the left cerebral hemisphere is representative of the global cerebral CAA assessment.

4.4.5.1 Left cerebral hemisphere parenchymal CAA and non-CAA SVD severity in first-ever lobar versus non-lobar ICH

Forty-five of the 55 (82%) participants with first-ever non-lobar ICH had moderate or severe non-CAA SVD and absent or mild parenchymal CAA in the left cerebral hemisphere (Figure 4.13). Nine (16%) had both moderate or severe non-CAA SVD and parenchymal CAA. One participant with a left lentiform nucleus ICH had no clear underlying cause of ICH (mild non-CAA SVD, absent parenchymal CAA and no macrovascular abnormality, coagulopathy or tumour).

Twenty-six of the 71 (37%) participants with first-ever lobar ICH had moderate or severe non-CAA SVD and absent or mild parenchymal CAA (Figure 4.13). Thirty-two (45%) had both moderate or severe non-CAA SVD and parenchymal CAA in the left cerebral hemisphere, and 11 (15%) had moderate or severe parenchymal CAA and absent or mild non-CAA SVD. Two (3%) participants had no clear underlying cause of ICH (absent or mild parenchymal CAA, mild non-CAA SVD and no macrovascular abnormality, coagulopathy or tumour).

Figure 4.13 Pathological severity of left cerebral hemisphere CAA and non-CAA SVD in first-ever ICH according to ICH location



CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage. SVD = small vessel disease.

4.4.5.2 Associations of left cerebral hemisphere CAA and vasculopathy in first-ever lobar ICH

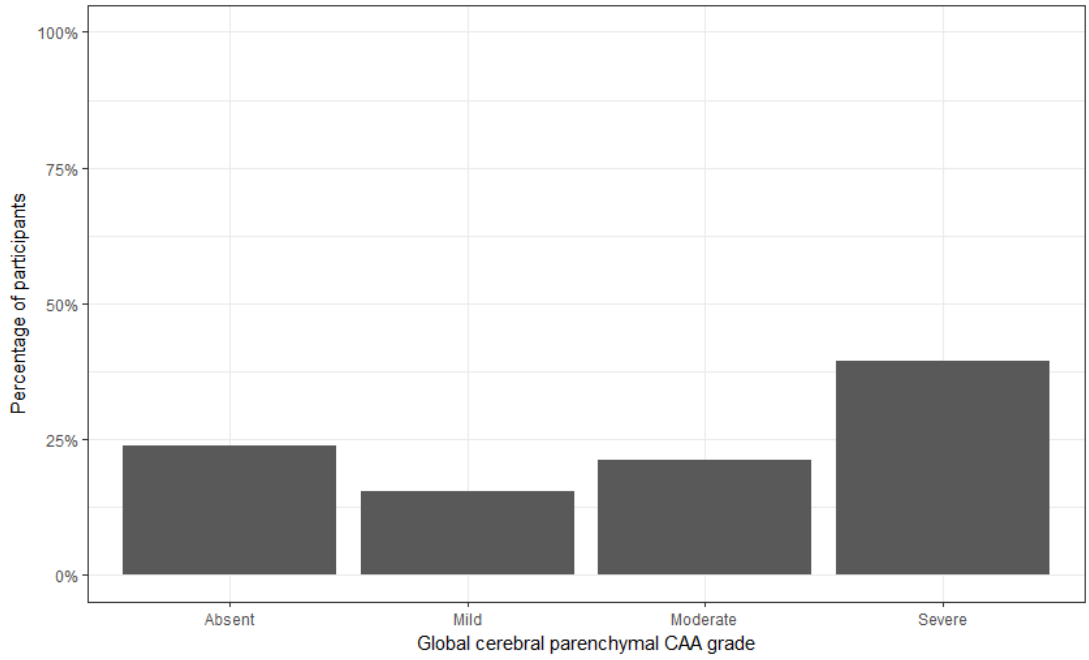
Given that CAA is associated with lobar but not non-lobar ICH,[153] and the low frequency of moderate or severe CAA in non-lobar ICH shown in Figure 4.13, I restricted my analyses assessing the associations of CAA to participants with first-ever lobar ICH.

Parenchymal CAA

The severity of left cerebral hemisphere parenchymal CAA in first-ever lobar ICH is shown in Figure 4.14. Twenty-eight participants had absent or mild parenchymal CAA while 43 had moderate or severe parenchymal CAA. Those with moderate or severe parenchymal CAA were significantly more likely to possess APOE ε2 or ε4 alleles, and moderate or severe meningeal CAA, capillary CAA, vasculopathy and more severe Braak stage and Thal phase on univariable assessment (Table 4.18). There were no statistically significant differences in age and pre-ICH co-morbidities between those with moderate or severe parenchymal CAA and those with absent or mild parenchymal CAA.

Moderate or severe left cerebral hemisphere parenchymal CAA was significantly associated with APOE ε4 allele possession when adjusting for APOE ε2 allele possession and Thal phase (Table 4.19).

Figure 4.14 Severity of left cerebral hemisphere parenchymal CAA in first-ever lobar ICH participants



CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

Table 4.18 Clinical and histopathological characteristics of first-ever lobar ICH participants stratified by left cerebral hemisphere parenchymal CAA severity

	Absent/mild parenchymal CAA (n=28)		Moderate/severe parenchymal CAA (n=43)		p value
Age (years); median (IQR)	83	(78-87)	81	(78-85)	0.423
Sex					
Female	14	(50)	29	(67)	0.142
Male	14	(50)	14	(33)	
Co-morbidities					
Hypertension	21	(75)	26	(61)	0.206
Ischaemic stroke*	4	(14)	6	(14)	1.000
Transient ischaemic attack*	2	(7)	3	(7)	1.000
Dementia*	2	(7)	10	(23)	0.108
Diabetes*	4	(14)	3	(7)	0.422
Atrial fibrillation	11	(39)	10	(23)	0.148
Myocardial infarction*	5	(18)	6	(14)	0.742
Hyperlipidaemia*	14	(14)	5	(12)	0.732
APOE ε4 carrier†	2	(7)	21	(49)	<0.001
APOE ε2 carrier†	3	(11)	15	(35)	0.027
Meningeal CAA					
Absent/mild	21	(75)	1	(2)	<0.001
Moderate/severe	7	(25)	42	(98)	
Capillary CAA	1	(4)	23	(54)	<0.001
Vasculopathy	0	(0)	33	(77)	<0.001
Non-CAA SVD					
Absent/mild	2	(7)	11	(26)	0.050
Moderate/severe	26	(93)	32	(74)	
Braak stage					
0-II	22	(77)	12	(28)	<0.001
III-IV	3	(11)	15	(35)	
V-VI	3	(11)	16	(37)	
Thal phase*					
0-I	14	(50)	8	(19)	<0.001
II-III	14	(50)	24	(56)	
IV-V	0	(0)	11	(26)	

Data are n (%) or median (IQR). * Fisher's exact test. † Data not available in 1 case with absent/mild CAA. APOE = apolipoprotein E. CAA=cerebral amyloid angiopathy. ICH = intracerebral haemorrhage. SVD=small vessel disease.

Table 4.19 Multivariable Firth's logistic regression model of first-ever lobar ICH associated with left cerebral hemisphere moderate or severe parenchymal CAA

	β Coefficient (standard error)		Odds ratio (95%CI)		p value
Intercept	-1.11	(0.53)	0.33	(0.09-0.86)	0.305
APOE ε4 carrier	1.71	(0.77)	5.51	(1.38-51.07)	0.027
APOE ε2 carrier	1.31	(0.74)	3.72	(0.95-24.29)	0.078
Thal phase					
0/I	--	--	Reference		
II/III	0.98	(0.61)	2.67	(0.84-10.49)	0.109
IV/V	3.01	(1.64)	20.20	(1.79-Not reached)	0.066

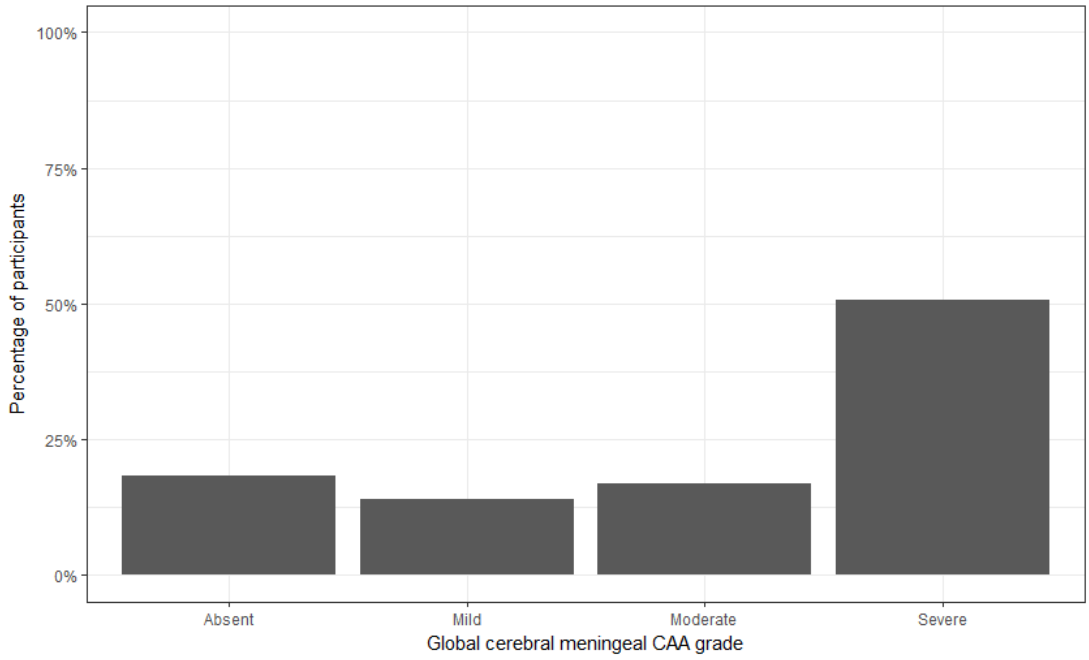
APOE = apolipoprotein E. CAA=cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

Meningeal CAA

The severity of left cerebral hemisphere meningeal CAA in first-ever lobar ICH is shown in Figure 4.15. Twenty-two participants with first-ever lobar ICH had absent or mild meningeal CAA in the left cerebral hemisphere while 49 had moderate or severe meningeal CAA. Those with moderate or severe meningeal CAA were more likely to have APOE ε4 allele possession, and moderate or severe parenchymal CAA, capillary CAA, vasculopathy and more severe Braak stage and Thal phase on univariable assessment (Table 4.20). There were no statistically significant differences in age and pre-ICH co-morbidities between those with moderate or severe meningeal CAA and those with absent or mild meningeal CAA.

APOE ε4, APOE ε2 and Thal phase were not independently associated with moderate or severe left cerebral hemisphere meningeal CAA (Table 4.21).

Figure 4.15 Severity of left cerebral hemisphere meningeal CAA in first-ever lobar ICH participants



CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

Table 4.20 Clinical and histopathological characteristics of first-ever lobar ICH participants stratified by left cerebral hemisphere meningeal CAA severity

	Absent/mild meningeal CAA (n=22)		Moderate/severe meningeal CAA (n=49)		p value
Age (years); median (IQR)	83	(76-85)	83	(79-86)	0.587
Sex					
Female	11	(50)	32	(65)	0.222
Male	11	(50)	17	(35)	
Co-morbidities					
Hypertension	17	(77)	30	(61)	0.186
Ischaemic stroke*	4	(18)	6	(12)	0.488
Transient ischaemic attack*	1	(5)	4	(18)	1.000
Dementia*	2	(9)	10	(20)	0.381
Diabetes*	4	(18)	3	(6)	0.192
Atrial fibrillation	9	(41)	12	(25)	0.161
Myocardial infarction*	4	(18)	7	(14)	0.729
Hyperlipidaemia*	3	(14)	6	(12)	1.000
APOE ε4 carrier†	3	(14)	20	(41)	0.030
APOE ε2 carrier†	3	(14)	15	(31)	0.152
Parenchymal CAA					
Absent/mild	21	(85)	7	(14)	<0.001
Moderate/severe	1	(5)	42	(86)	
Capillary CAA	1	(5)	23	(47)	<0.001
Vasculopathy	0	(0)	33	(67)	<0.001
Non-CAA SVD*					
Absent/mild	3	(14)	10	(20)	0.741
Moderate/severe	19	(86)	39	(80)	
Braak stage					
0-II	17	(77)	17	(35)	0.004
III-IV	3	(14)	15	(31)	
V-VI	2	(9)	17	(35)	
Thal phase*					
0-I	11	(50)	11	(22)	<0.009
II-III	11	(50)	27	(55)	
IV-V	0	(0)	11	(22)	

Data are n (%) or median (IQR). * Fisher's exact test. † Data not available in 1 case with absent/mild CAA. APOE = apolipoprotein E. CAA=cerebral amyloid angiopathy. ICH = intracerebral haemorrhage. SVD=small vessel disease.

Table 4.21 Multivariable Firth's logistic regression model of first-ever lobar ICH associated with left cerebral hemisphere moderate or severe meningeal CAA

	β Coefficient (standard error)		Odds ratio (95%CI)		p value
Intercept	-0.26	(0.47)	0.77	(0.29-1.88)	0.577
APOE ε4 carrier	0.65	(0.70)	1.91	(0.52-10.72)	0.353
APOE ε2 carrier	0.72	(0.71)	2.06	(0.57-11.92)	0.305
Thal phase					
0/I	--	--	Reference		
II/III	0.84	(0.58)	2.33	(0.77-7.93)	0.145
IV/V	2.69	(1.57)	14.68	(1.41-Not reached)	0.086

APOE = apolipoprotein E. CAA=cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

Capillary CAA

Forty-seven participants with first-ever lobar ICH had no capillary CAA in the left cerebral hemisphere while 24 had capillary CAA present. Those with capillary CAA were more likely to have APOE ε2 and ε4 allele possession, and moderate or severe parenchymal CAA, moderate or severe meningeal CAA, vasculopathy and more severe Braak stage and Thal phase on histopathological assessment on univariable assessment (Table 4.22). Those without capillary CAA had more frequent pre-ICH hypertension. There were no other statistically significant differences in age and pre-ICH co-morbidities between those with and without capillary CAA.

APOE ε4, APOE ε2 and Thal phase were not independently associated with capillary CAA (Table 4.23).

Table 4.22 Clinical and histopathological characteristics of first-ever lobar ICH participants stratified by the left cerebral hemisphere capillary CAA

	Absent capillary CAA (n=47)		Present capillary CAA (n=24)		p value
Age (years); median (IQR)	82	(78-86)	82	(78-86)	0.763
Sex					
Female	28	(60)	15	(63)	0.811
Male	19	(40)	9	(38)	
Co-morbidities					
Hypertension	36	(77)	11	(46)	0.010
Ischaemic stroke*	8	(17)	2	(8)	0.477
Transient ischaemic attack*	4	(9)	1	(4)	0.656
Dementia*	6	(13)	6	(25)	0.315
Diabetes*	5	(11)	2	(8)	1.000
Atrial fibrillation	16	(34)	5	(21)	0.249
Myocardial infarction*	9	(19)	2	(8)	0.312
Hyperlipidaemia*	9	(19)	0	(0)	0.024
APOE ε4 carrier†	10	(22)	13	(54)	0.006
APOE ε2 carrier†	8	(17)	10	(42)	0.027
Parenchymal CAA					
Absent/mild	27	(57)	1	(4)	<0.001
Moderate/severe	20	(43)	23	(96)	
Meningeal CAA					
Absent/mild	21	(45)	1	(4)	<0.001
Moderate/severe	26	(55)	23	(96)	
Vasculopathy	14	(30)	19	(79)	<0.001
Non-CAA SVD*					
Absent/mild	8	(17)	5	(21)	0.751
Moderate/severe	39	(83)	19	(79)	
Braak stage					
0-II	28	(60)	6	(25)	0.015
III-IV	8	(17)	10	(42)	
V-VI	11	(23)	8	(33)	
Thal stage*					
0-I	19	(40)	3	(13)	0.016
II-III	24	(51)	14	(58)	
IV-V	4	(9)	7	(29)	

Data are n (%) or median (IQR). * Fisher's exact test. † Data not available in 1 case with absent/mild CAA. APOE = apolipoprotein E. CAA=cerebral amyloid angiopathy. ICH = intracerebral haemorrhage. SVD=small vessel disease.

Table 4.23 Multivariable logistic regression model of first-ever lobar ICH associated with left cerebral hemisphere capillary CAA

	β Coefficient (standard error)		Odds ratio (95%CI)		p value
Intercept	-2.39	(0.70)	0.09	(0.02-0.36)	<0.001
APOE ε4 carrier	0.97	(0.61)	2.63	(0.80-8.69)	0.113
APOE ε2 carrier	1.17	(0.63)	3.23	(0.94-11.08)	0.062
Thal phase					
0/I	--	--	Reference		
II/III	1.25	(0.75)	3.50	(0.81-15.12)	0.093
IV/V	1.89	(0.97)	6.61	(0.99-44.09)	0.051

APOE = apolipoprotein E. CAA=cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

Vasculopathy

Thirty-eight participants with first-ever lobar ICH had no vasculopathy in the left cerebral hemisphere while 33 had vasculopathy present. Those with vasculopathy were more likely to have a pre-ICH diagnosis of dementia, lobar ICH location and APOE ε4 allele possession, and moderate or severe parenchymal CAA, moderate or severe meningeal CAA, capillary CAA, and more severe Braak stage and Thal phase on univariable assessment (Table 4.24). There were no statistically significant differences in age between those with and without vasculopathy

Left cerebral hemisphere vasculopathy was significantly associated with Thal phase when adjusting for APOE ε2 and ε4 allele possession (Table 4.25).

Table 4.24 Clinical and histopathological characteristics of first-ever lobar ICH participants stratified by left cerebral hemisphere vasculopathy

	Absent vasculopathy (n=38)		Vasculopathy present (n=33)		p value
Age (years); median (IQR)	83	(79-87)	81	(76-85)	0.321
Sex					
Female	24	(63)	19	(58)	0.631
Male	14	(37)	14	(42)	
Co-morbidities					
Hypertension	29	(76)	18	(55)	0.052
Ischaemic stroke*	6	(16)	4	(12)	0.742
Transient ischaemic attack*	3	(8)	2	(6)	1.000
Dementia	3	(8)	9	(27)	0.030
Diabetes*	5	(13)	2	(6)	0.438
Atrial fibrillation	14	(37)	7	(21)	0.150
Myocardial infarction	7	(18)	4	(12)	0.464
Hyperlipidaemia*	6	(16)	3	(9)	0.489
APOE ε4 carrier†	7	(19)	16	(49)	0.009
APOE ε2 carrier†	6	(16)	12	(36)	0.054
Parenchymal CAA					
Absent/mild	28	(74)	0	(0)	<0.001
Moderate/severe	10	(26)	33	(33)	
Meningeal CAA					
Absent/mild	22	(58)	0	(0)	<0.001
Moderate/severe	16	(42)	33	(33)	
Capillary CAA	5	(13)	19	(58)	<0.001
Non-CAA SVD					
Absent/mild	6	(16)	7	(21)	0.556
Moderate/severe	32	(84)	26	(79)	
Braak stage					
0-II	28	(74)	6	(18)	<0.001
III-IV	5	(13)	13	(39)	
V-VI	5	(13)	14	(42)	
Thal stage					
0-I	19	(50)	3	(9)	<0.001
II-III	19	(50)	19	(58)	
IV-V	0	(0)	11	(33)	

Data are n (%). * Fisher's exact test. † Data not available in 1 case with absent/mild CAA. APOE = apolipoprotein E. CAA=cerebral amyloid angiopathy. ICH = intracerebral haemorrhage. SVD=small vessel disease.

Table 4.25 Multivariable Firth's logistic regression model of first-ever lobar ICH associated with left cerebral hemisphere vasculopathy

	β Coefficient (standard error)		Odds ratio (95%CI)		p value
Intercept	-2.09	(0.66)	0.12	(0.02-0.38)	0.002
APOE ε4 carrier	0.46	(0.65)	1.58	(0.42-6.19)	0.482
APOE ε2 carrier	1.10	(0.69)	3.00	(0.82-15.01)	0.109
Thal phase					
0/I	--	--	Reference		
II/III	1.72	(0.71)	5.61	(1.60-35.75)	0.014
IV/V	4.58	(1.63)	97.08	(8.31-Not reached)	0.005

APOE = apolipoprotein E. CAA=cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

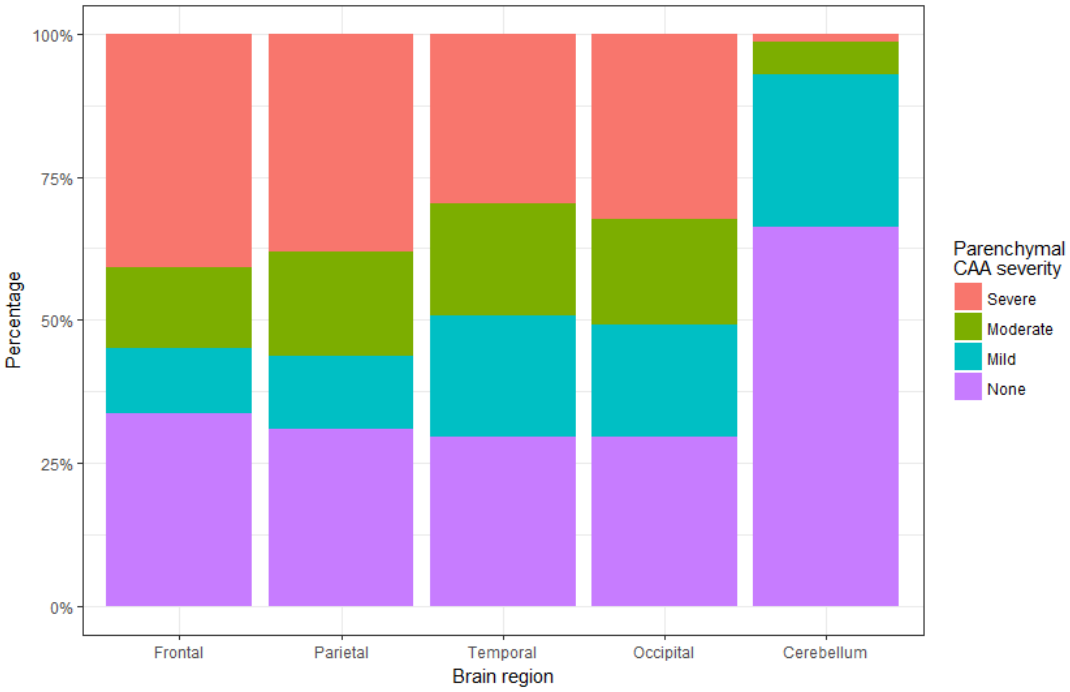
4.4.6 Pathological distribution of CAA in first-ever SVD-associated lobar ICH

4.4.6.1 Parenchymal CAA

The distribution and severity of global parenchymal CAA in 71 participants with first-ever lobar ICH is shown in Figure 4.16. Moderate or severe parenchymal CAA was present in the frontal lobes in 39 (55%) participants, the parietal lobes in 40 (56%), temporal lobes in 35 (49%), and the occipital lobes in 36 (51%) participants. In contrast, only five (7%) participants had moderate or severe parenchymal CAA in the cerebellum.

51% of participants had moderate or severe parenchymal CAA in the occipital lobes while 54% had moderate or severe parenchymal CAA in the other cerebral lobes (frontal, parietal and temporal). The risk ratio of moderate or severe parenchymal CAA in the occipital lobes compared with the other cerebral lobes was 0.95 (95% CI 0.73-1.23).

Figure 4.16 Distribution and severity of parenchymal CAA in first-ever lobar ICH participants



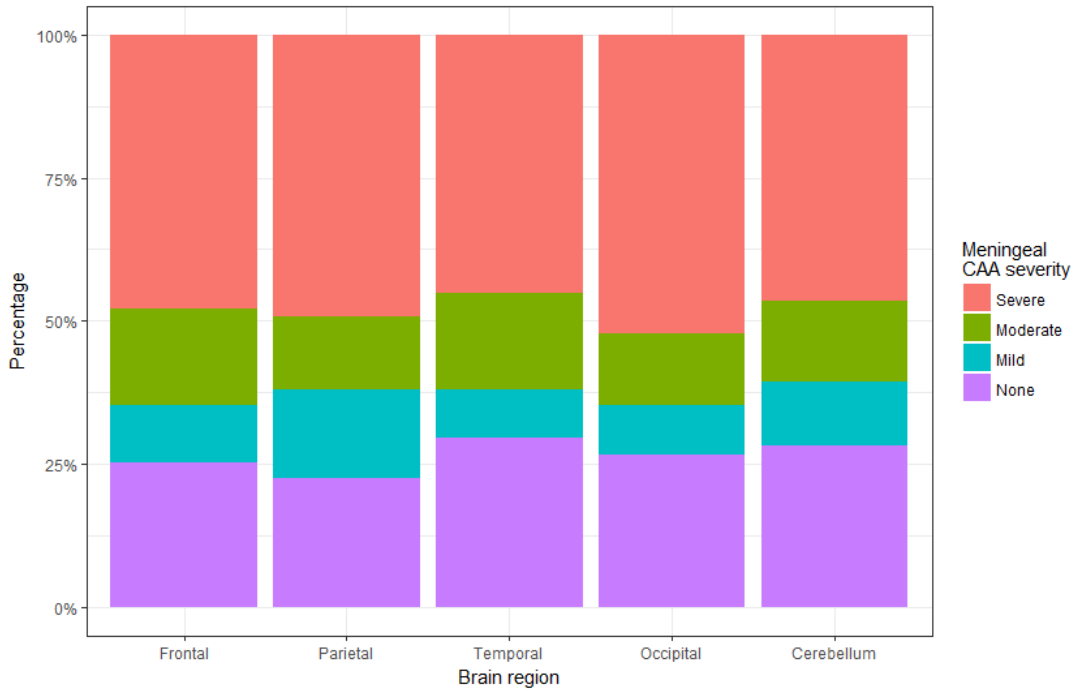
CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

4.4.6.2 Meningeal CAA

The distribution and severity of global cerebral meningeal CAA in participants with first-ever lobar ICH is shown in Figure 4.17. Moderate or severe meningeal CAA was present in the frontal lobes in 46 (65%) participants, the parietal lobes in 44 (62%), temporal lobes in 44 (62%), and the occipital lobes in 46 (65%) participants. Forty-three (61%) participants had moderate or severe meningeal CAA in the cerebellum.

65% of participants had moderate or severe meningeal CAA in the occipital lobes while 63% had moderate or severe meningeal CAA in the other cerebral lobes (frontal, parietal and temporal). The risk ratio of moderate or severe meningeal CAA in the occipital lobes compared with the other cerebral lobes was 1.03 (95%CI 0.84-1.26).

Figure 4.17 Distribution and severity of meningeal CAA in first-ever lobar ICH participants



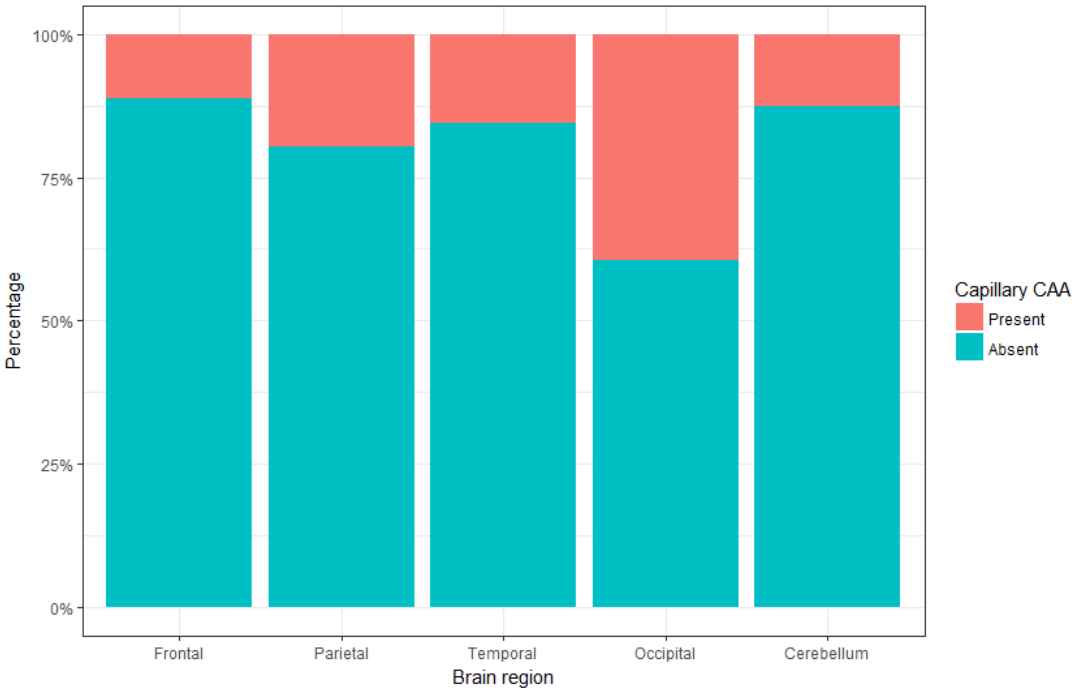
CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

4.4.6.3 Capillary CAA

The distribution of global cerebral capillary CAA in participants with first-ever lobar ICH is shown in Figure 4.18. Capillary CAA was present in the frontal lobes in 8 (11%) participants, the parietal lobes in 14 (20%), temporal lobes in 11 (15%), and the occipital lobes in 28 (39%) participants. Nine (13%) participants had capillary CAA in the cerebellum.

39% of participants had capillary CAA in the occipital lobes while 15% had capillary CAA in the other cerebral lobes (frontal, parietal and temporal). The risk ratio of capillary CAA in the occipital lobes compared with the other cerebral lobes was 2.55 (95% CI 1.66-3.90).

Figure 4.18 Distribution of capillary CAA in first-ever lobar ICH participants



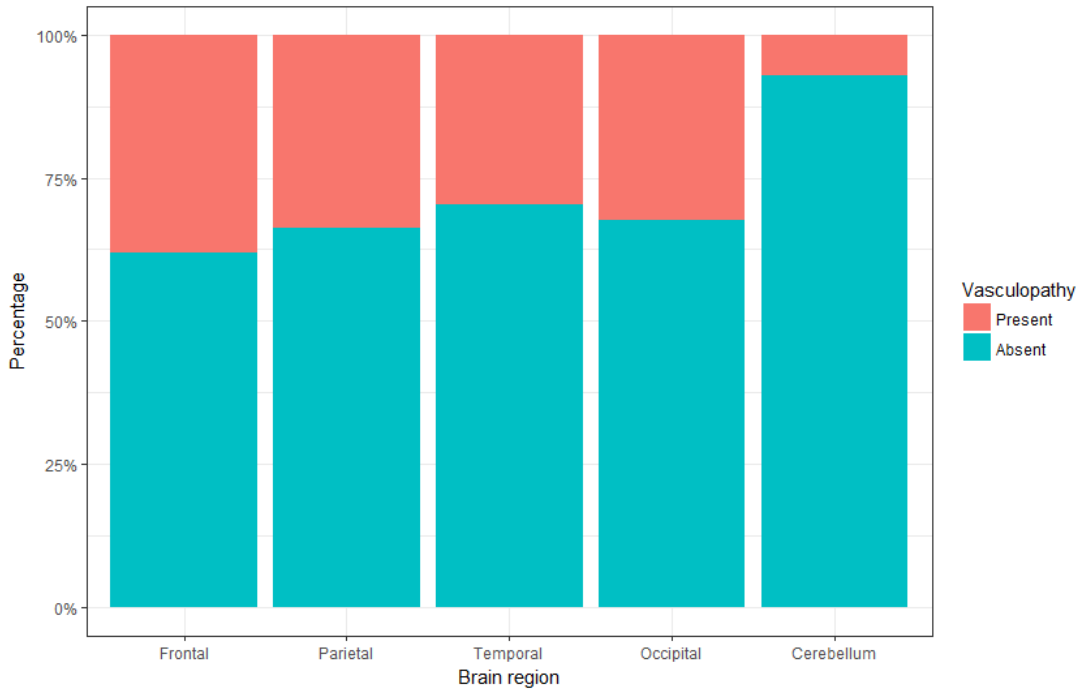
CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

4.4.6.4 Vasculopathy

The distribution of global cerebral vasculopathy in participants with first-ever lobar ICH is shown in Figure 4.19. Vasculopathy was present in the frontal lobes in 27 (38%) participants, the parietal lobes in 24 (34%), temporal lobes in 21 (30%), and the occipital lobes in 23 (32%) participants. Five (7%) participants had vasculopathy in the cerebellum.

32% of participants had vasculopathy in the occipital lobes while 34% had vasculopathy in the other cerebral lobes (frontal, parietal and temporal). The risk ratio of vasculopathy in the occipital lobes compared with the other cerebral lobes was 0.96 (95% CI 0.65-1.41).

Figure 4.19 Distribution of vasculopathy in first-ever lobar ICH participants

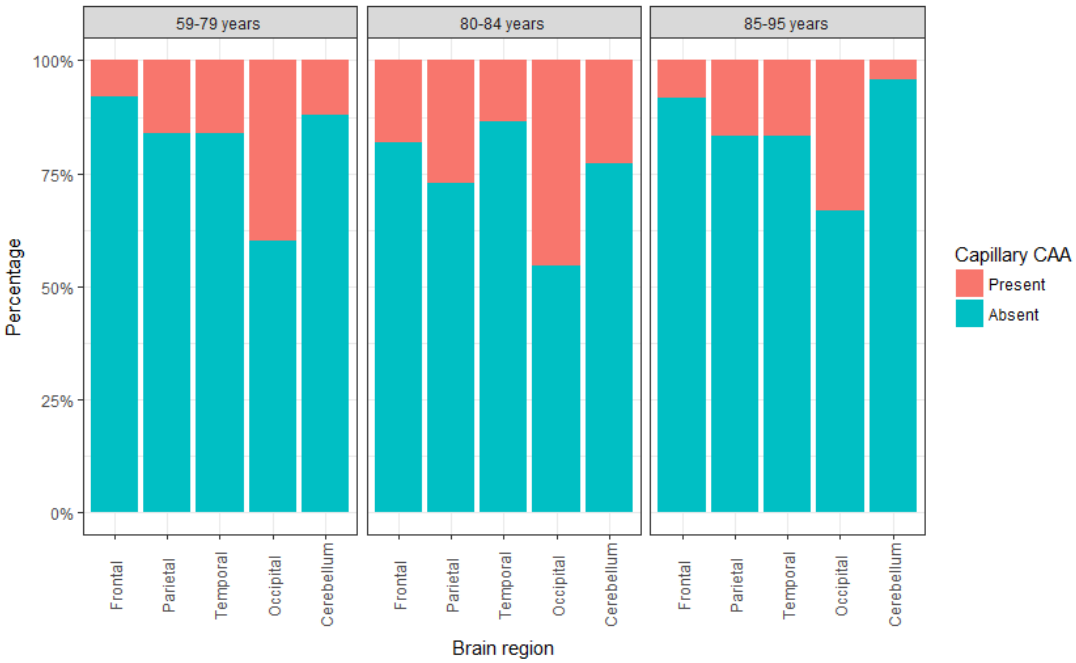


ICH = intracerebral haemorrhage.

4.4.6.5 Sensitivity analyses

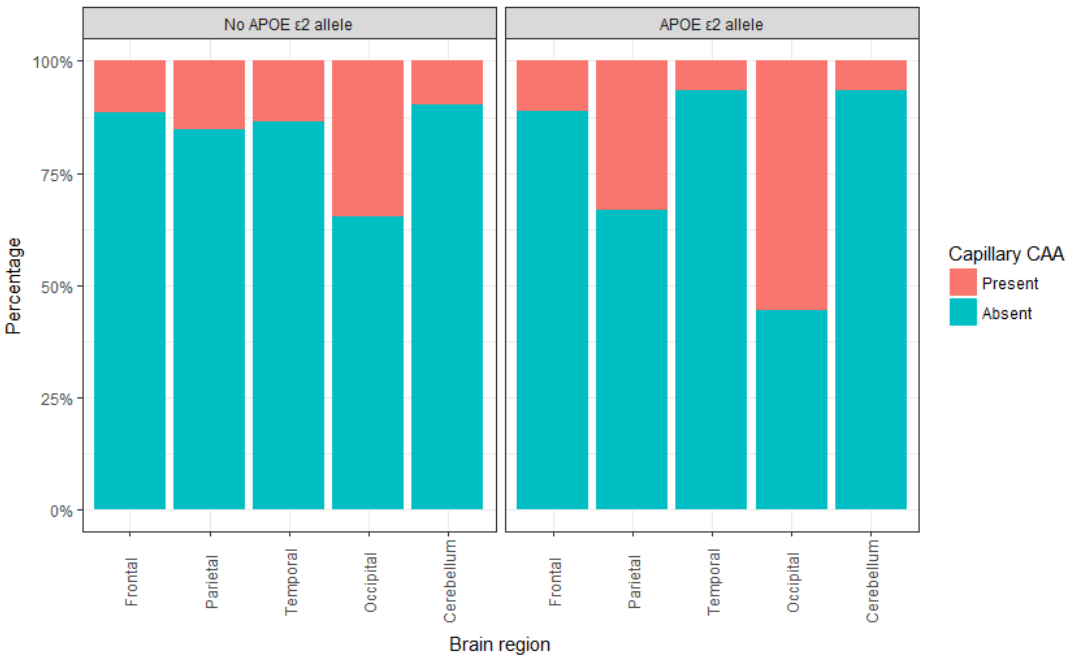
The occipital predominance of capillary CAA persisted regardless of age (Figure 4.20), APOE ε2 genotype (Figure 4.21) or APOE ε4 genotype (Figure 4.22). The strength of the occipital capillary CAA predominance seemed to increase with increasing Thal phase. The risk ratio of capillary CAA presence in the occipital lobes compared with the other cerebral lobes was 1.68 (95%CI 0.62-4.45) among the 22 participants with Thal phase 0 or I, 2.37 (95%CI 1.34-4.18) among the 38 participants with Thal phase II or III and 4.80 (95% CI 1.98-11.63) among the 11 participants with Thal phase IV or V (Figure 4.23).

Figure 4.20 Distribution of capillary CAA in first-ever lobar ICH participants stratified by age at the time of the index ICH



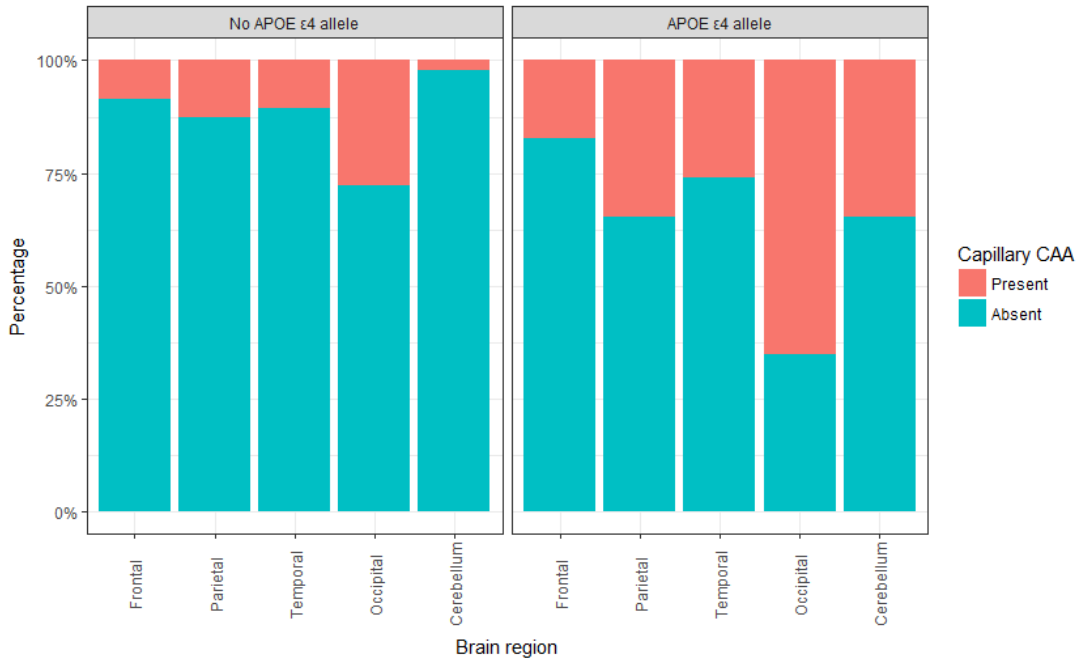
CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

Figure 4.21 Distribution of capillary CAA in first-ever lobar ICH participants stratified by APOE ε2 allele possession



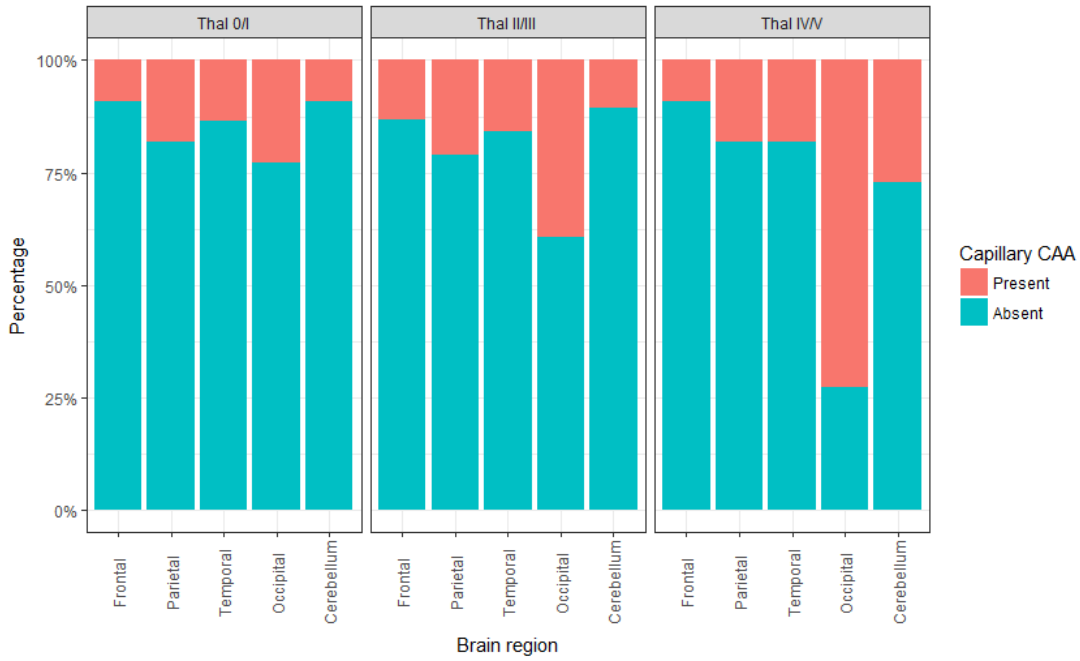
APOE = apolipoprotein E. CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

Figure 4.22 Distribution of capillary CAA in first-ever lobar ICH participants stratified by APOE ε4 allele possession



APOE = apolipoprotein E. CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

Figure 4.23 Distribution of capillary CAA in first-ever lobar ICH participants stratified by Thal phase



CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage.

There was no occipital predominance for moderate or severe parenchymal or meningeal CAA or vasculopathy when stratifying the analyses by age, APOE ε2 or ε4 genotype or Thal phase (data not shown).

The severity of parenchymal or meningeal CAA and the presence of capillary CAA or vasculopathy did not vary between the cerebral hemisphere or lobe affected by ICH compared with the contralateral unaffected cerebral hemisphere or lobe (data not shown).

4.5 Discussion

4.5.1 Main findings

- Assessment of selection bias during the community-based LATCH cohort study
    - Those with a first-ever SVD-associated ICH who consented to research brain autopsy were similar to non-consenters apart from having less frequent pre-ICH ischaemic stroke or myocardial infarction, and more frequent lobar ICH locations and subarachnoid haemorrhage.
    - Those with a first-ever SVD-associated ICH who had a research brain autopsy were older with more frequent pre-ICH dementia and more severe pre-ICH disability than the rest of the LATCH cohort. They also had larger ICHs and more frequent subarachnoid haemorrhage.
- Diagnostic accuracy of histopathological assessment of CAA and vasculopathy in the left cerebral hemisphere versus systematic whole brain autopsy
    - Histopathological assessment of parenchymal CAA, meningeal CAA and vasculopathy using the left cerebral hemisphere had excellent sensitivity and specificity (≥97%) compared with the global cerebral histopathological assessment.

  - Histopathological assessment for the presence of capillary CAA in the left cerebral hemisphere had moderate sensitivity (82%) compared with global cerebral histopathological assessment.
- Diagnostic accuracy of histopathological assessment of CAA and vasculopathy in the cerebral lobe affected by ICH (simulated cortical biopsy) versus systematic whole brain autopsy
  - Histopathological assessment of parenchymal CAA and meningeal CAA using the Love et al. grading system[36] in the cerebral lobe affected by ICH had good sensitivity and excellent specificity compared with the global cerebral histopathological assessment, regardless of the age at the time of index ICH.
  - Histopathological assessment for the presence of vasculopathy in the cerebral lobe affected by ICH had modest sensitivity, while the sensitivity for capillary CAA presence was poor.
  - The presence of any CAA (Vonsattel grade ≥1) was 100% sensitive compared with the global cerebral histopathological assessment. The specificity was modest (70%) and this decreased with age.
  - The presence of complete replacement of a vessel wall with amyloid-β (Vonsattel grade ≥2) was 95% sensitive and 79% specific compared with global cerebral histopathological assessment. Again, the specificity decreased with age.
- Pathological severity and associations of SVDs in first-ever SVD-associated ICH
  - 98% of participants with a non-lobar ICH had moderate or severe non-CAA SVD.
  - 45% of participants with a lobar ICH had mixed moderate or severe CAA and non-CAA SVD, 37% had moderate or severe non-CAA alone, and 15% had moderate or severe CAA alone.
  - Moderate or severe parenchymal CAA was significantly associated with APOE ε4 allele possession when adjusting for APOE ε2 genotype and Thal phase.

    - The presence of vasculopathy was independently associated with Thal phase but not APOE ε2 or ε4 genotype.
- Pathological distribution of CAA in first-ever lobar ICH
    - There was no occipital predominance for parenchymal CAA, meningeal CAA or vasculopathy regardless of age, APOE genotype or Thal phase.
    - Capillary CAA showed an occipital predominance irrespective of age, APOE genotype or Thal phase.

4.5.2 Strengths of the study

The LINCHPIN brain bank is a unique dataset to study the histopathology of SVD-associated ICH.

The RUSH team minimised selection bias by prospectively inviting all eligible participants to the study, regardless of clinical characteristics, imaging features or genotype. There were many similarities between ICH patients who consented to a research brain autopsy and non-consenters, as well as those who had a research brain autopsy versus non-donors.

We minimised information bias through the use of a standardised approach for systematically acquiring the research brain autopsy[217] and assessing histopathological features of SVDs and Alzheimer's pathology using published scales.[36, 219, 220] The histopathological assessors were masked to the clinical, imaging and genetic features. Finally, the amount of missing data was low, with only one participant not having APOE genotyping performed.

4.5.3 Weaknesses of the study

Although we tried to limit selection bias, those included in the study were older with more frequent pre-ICH dementia and more severe pre-ICH disability than those not included. They also had larger ICHs and more frequent subarachnoid haemorrhage. The older age and larger ICH volume of included participants probably reflect the increased likelihood of dying after ICH in such patients.[244] The more frequent subarachnoid haemorrhage is

probably related to the increased proportion of lobar ICH and the larger ICH volume of included participants, both of which are associated with subarachnoid haemorrhage.[40, 237]

The time between ICH onset and autopsy was prolonged for some participants. The autopsy occurred over one year after the ICH for 25 participants (20%) and over three years for eight participants (6%). Given that CAA is a progressive, age-related process, its severity may have increased in those with a long-delay between ICH and autopsy. I did not have sufficient power to adjust my analyses for the time between ICH and autopsy. However, the time interval between ICH and autopsy in most participants was short (median 12 days, IQR 6-162 days), making it easier to relate histopathological changes at autopsy to those likely present at the time of the index ICH.

The sample size was modest, with 126 participants with first-ever SVD-associated ICH, 71 of whom had a lobar ICH. The sample size limited the number of variables I could include in the multivariable analyses of CAA and vasculopathy. For example, I did not have sufficient power to adjust for age and sex. However, this is one of the largest brain banks of SVD-associated ICH worldwide. In comparison, the original Boston criteria were validated in 39 lobar ICH participants who had brain tissue sampled. Only 14 of these participants had tissue from a full brain autopsy.[103] The modified Boston criteria were derived using data from 60 participants, 11 of whom did not have ICH. Only 19 of the participants had brain tissue from a full brain autopsy.[110]

It was not possible to mask the histopathological assessors to the ICH location. Furthermore, the assessors were not masked to the location of the pathological samples. This may have biased assessment of CAA and non-CAA SVD presence and severity.

It would be interesting to assess the associations of CAA in non-lobar ICH in more detail. However, the CAA ratings were not performed in the basal ganglia, thalami or brainstem because previous studies have shown these

regions are rarely affected by CAA.[60, 267, 270] I was therefore unable to assess the frequency and severity of CAA in locations where most of the non-lobar ICHs occurred. Also, the severity of non-CAA SVD was quantified as an overall score in the left cerebral hemisphere rather than assessed separately in individual brain regions as it is usually considered to be a symmetrical process. Therefore I could not assess the distribution and severity of non-CAA SVD, which again would be interesting.

I simulated cortical biopsies by using the ratings from the cerebral lobe affected by the index ICH. However, the histopathological ratings were based on sections from a tissue block taken at autopsy, rather than a tissue sample with the size and shape that is obtained by cortical biopsy. As a result, the amount of tissue available for histopathological assessment was probably larger than is available from a cortical biopsy. Also, the simulated biopsy samples were assessed along with sections from the other brain regions obtained at autopsy, rather than in isolation, as would occur with a cortical biopsy. These factors could increase the apparent diagnostic accuracy of the simulated cortical biopsies.[176]

4.5.4 Comparison with other studies

4.5.4.1 Selection bias

The participants included in research studies should be representative of the population of interest to ensure the results are generalisable.[271] This is particularly important for brain autopsy studies given the declining autopsy rates.[272] Most previous autopsy studies of CAA did not provide an assessment of selection bias. The nesting of the LINCHPIN study within the community-based LATCH cohort study of ICH allows an accurate assessment of selection bias. Those who consented to research brain autopsy were generally similar to non-consenters, although those who underwent research brain autopsy differed from the rest of the cohort as described in 4.4.2.2. Many of these differences are likely to reflect their associations with death soon after ICH[244] and are thus difficult to avoid in

an autopsy study. However, being able to quantify the selection bias is important as it indicates to whom the results are generalisable.

4.5.4.2 Diagnostic accuracy of simulated cortical biopsies for CAA-associated lobar ICH

A previous study assessed the diagnostic accuracy of simulated cortical biopsy from brain autopsies for CAA-associated ICH.[164] Vonsattel grade ≥1 had a sensitivity of 100% and a specificity ranging from 95% for those aged 65-74 years to 77% for those ≥85 years. Using Vonsattel grade ≥2 as the cut-off resulted in a sensitivity of 93% and specificity ranging from 98% for those aged 65-74 years to 88% for those ≥85 years. The absence of amyloid-β in the simulated cortical biopsy was said to show strong evidence against CAA-associated ICH. However, their study had several methodological limitations. There were only two participants with CAA-associated ICH (defined as corticosubcortical ICH with severe CAA) and no participants with non-CAA-associated ICH. Fourteen simulated cortical biopsies were assessed from each of the two CAA-associated ICH participants. The authors treated these individual simulated biopsies as independent samples and used them to calculate the sensitivity. The specificity was calculated as 100% minus the percent likelihood of encountering the same degree of CAA in a brain sample from the general elderly population. These limitations make the results difficult to interpret.

In contrast, I included 71 participants with lobar ICH, 43 of whom were classified as CAA-associated lobar ICH (moderate or severe global cerebral parenchymal CAA) and 28 as non-CAA–associated lobar ICH (absent or mild global cerebral parenchymal CAA). I calculated the sensitivity and specificity using standard approaches. Vonsattel grade ≥1 in the cerebral lobe affected by ICH had a sensitivity of 100% (95%CI 90-100) and a specificity of 71% (95%CI 51-86) for moderate or severe global cerebral parenchymal CAA. The specificity ranged from 90% in those aged 56-79 years to 50% in those aged 85-95 years (Table 4.15). Vonsattel grade ≥2 had a sensitivity of 95% (95%CI 83-99) and a specificity of 79% (95%CI 59-91). The specificity

ranged from 90% in those aged 56-79 years to 67% in those aged 85-95 years (Table 4.17).

My data are consistent with the prior work.[164] Together they suggest that cortical biopsy is highly sensitive for moderate or severe CAA, and Vonsattel grade <1 can be used to rule out CAA-associated ICH. The specificity of cortical biopsy for moderate or severe CAA decreases with age, and is highest in those aged age younger than 85 years and when using Vonsattel grade ≥2 as the positive cut off.

4.5.4.3 Severity and associations of CAA in first-ever SVD-associated ICH

Moderate or severe non-CAA SVD was present in all but one (98%) participants with first-ever non-lobar ICH. This finding is consistent with previous studies that have shown that non-lobar ICH, in particular those occurring in the basal ganglia and thalamus, are caused by arteriolosclerosis.[232, 273] Sixteen percent of participants with non-lobar ICH also had moderate or severe parenchymal CAA in the left cerebral hemisphere, which is similar to the population prevalence of CAA in this age group.[14]

The most frequent SVD associated with first-ever lobar ICH in my study was non-CAA SVD, with moderate or severe non-CAA SVD occurring in 58 out of 71 (82%) participants with lobar ICH. In contrast, 43 of the 71 participants (61%) with lobar ICH had moderate or severe parenchymal CAA. CAA and non-CAA SVDs frequently coexist in first-ever lobar ICH, with 45% of lobar ICH participants having both moderate or severe parenchymal CAA and non-CAA SVD. Only a minority have “pure CAA” (15% with moderate or severe parenchymal CAA and mild non-CAA SVD) or “pure non-CAA SVD” (37% with moderate or severe non-CAA SVD and absent or mild parenchymal CAA). Previous retrospective autopsy studies of CAA-associated ICH have shown the coexistence of CAA and non-CAA SVDs pathologies, with up to 57% of patients having both CAA and arteriolosclerosis.[214, 274-276] Therefore, in the context of lobar ICH, CAA and non-CAA SVDs should not

be considered as mutually exclusive disease processes. Whether and how CAA and non-CAA SVDs interact in the context of ICH remains to be established.

I identified an independent association between moderate or severe parenchymal CAA and APOE ε4 genotype, which is in keeping with previous meta-analyses.[25, 254] APOE ε2 was more common in lobar ICH with moderate or severe parenchymal CAA, but this did not reach statistical significance (OR 3.72, 95% CI 0.95 to 24.29, p=0.078). There was no association between moderate or severe meningeal CAA, capillary CAA or vasculopathy and APOE genotype. The lack of significant association between capillary CAA and APOE ε2 and ε4 contradicts previous autopsy studies,[257, 277] which may reflect differences in the populations (ICH versus dementia/non-ICH) or the limited power in my study.

There was no independent association between parenchymal CAA, meningeal CAA, capillary CAA or vasculopathy and Thal phase. Many previous studies have demonstrated an association between CAA and Alzheimer's disease neuropathological changes.[278] However, the frequency of Alzheimer's disease pathology has been shown to be lower in those with more severe CAA.[270] This may explain the findings in my study. Alternatively, the lack of an association may reflect the limited power in my multivariable analyses, especially as Thal phase IV/V showed a borderline significant association in all analyses.

4.5.4.4 Distribution of CAA in lobar ICH

It is commonly cited that the occipital lobe is the brain region most frequently and severely affected by CAA.[18, 258] The greater frequency of CAA in the occipital lobes has been used to explain a variety of neuroimaging findings in patients with CAA, such as the spatial clustering of haemorrhages,[208] reductions in brain connectivity[259] and vascular reactivity,[260] WMH distribution[261] and amyloid PET tracer uptake.[252, 262] However, the previous neuropathological studies assessing the distribution of CAA have

shown that parenchymal and meningeal CAA is generally widely distributed throughout the cerebral lobes without a consistent occipital predominance.

Vinters et al. assessed the distribution of CAA in the cerebral cortex using Congo red in a hospital-based autopsy study regardless of clinical diagnosis.[28] They found that in 30 patients aged over 60 years, CAA of any severity was present in the occipital lobes in 65% of patients compared with 44% in the other cerebral lobes (RR 1.48 (95%CI 1.16-1.90)). Severe CAA was present in the occipital lobes in 28% compared with 18% in the other cerebral lobes (RR 1.59 (95%CI 0.96.16-2.66)). Tomonaga assessed the distribution of CAA using Congo red in a non-selective hospital-based autopsy study in Tokyo, Japan. Seven patients of out 128 clinical autopsies aged 60-105 had overall severe CAA.[263] Moderate or severe CAA (defined as CAA involving at least 3 out of 10 vessels) was present in the occipital lobe in 100% compared with 86% in the other cerebral lobes (RR1.17 (95%CI 0.98-1.39)). Neither of these studies reported the distribution of meningeal or capillary CAA.

Nelson et al. performed a prospective autopsy study of normal ageing and Alzheimer's disease in Kentucky, USA.[264] Parenchymal and meningeal CAA was assessed in the four cerebral lobes using immunohistochemistry in 371 brains. Greater prevalence of severe parenchymal and meningeal CAA was found in the occipital lobes (19% and 41% respectively), compared with the other cerebral lobes (frontal, 9% and 21%; parietal, 12% and 23%; temporal, 11% and 23%).

Xu et al. performed a retrospective hospital-based neuropathological study of 362 autopsies of patients aged 60 to 95 years regardless of clinical diagnosis in Beijing, China.[265] CAA was identified in 114 cases using Congo red and immunohistochemistry, 27 of whom had severe CAA. The frontal lobe was most frequently affected by severe CAA (96%), followed by the occipital lobe (89%), parietal lobe (63%) and cerebellum (52%). The type of vessel involved was not specified.

Masuda et al. performed a prospective population-based autopsy study in Hisayama, Japan between 1971 and 1983 regardless of clinical diagnosis.[266] They identified CAA using Congo red in 91 out of 400 cases. CAA of any severity was present in the occipital lobes in 54% compared with 65% in the other cerebral lobes (RR 0.83 (95%CI 0.67-1.02)). The type of vessel involved was not specified.

In my community-based neuropathological study of first-ever lobar ICH, I found that parenchymal CAA, meningeal CAA and vasculopathy are all relatively evenly distributed throughout the cerebral lobes, even after accounting for participant age at the time of the index ICH, APOE genotype and Thal phase.

In contrast, I found a strong occipital predominance for capillary CAA. This occurred regardless of age, APOE genotype or Thal phase. A recent large prospective population-based, non-selective neuropathological study also showed that capillary CAA was most frequently found in the occipital lobes.[279] The significance of this occipital predominance of capillary CAA is uncertain, especially in the context of ICH and the distribution of imaging findings previously described in CAA.[208, 252, 259-262]

A recent phase 2 randomised controlled trial assessed the efficacy of immunotherapy with ponezumab, a monoclonal antibody against β-amyloid$_{1-40}$, in patients with probable CAA according to the modified Boston criteria.[280] The primary outcome was change in the cerebrovascular reactivity in the visual cortex at 90 days. The hypothesis was that a treatment response to ponezumab would decrease vascular amyloid and improve vascular reactivity. However, the treatment group had a trend towards reduced cerebrovascular reactivity, which was opposite to the hypothesized direction.

The reasons for this result are unclear. It may indicate that vascular amyloid was not sufficiently cleared, that clearance of vascular amyloid does not improve vasoreactivity or could even cause vascular damage, or the time point was too early.

Other possible explanations relate to the choice of occipital cerebrovascular reactivity as the outcome. Impaired occipital cerebrovascular in CAA is based on a few small case control studies, many of which used inappropriate control groups.[260, 281-283] Therefore, its validity as a biomarker of CAA is unclear. Furthermore, the pathophysiology of impaired occipital vascular reactivity in CAA is not understood. As discussed above, decreased occipital cerebrovascular reactivity is thought to reflect the occipital CAA predominance. However, given the lack occipital gradient for parenchymal and meningeal CAA and vasculopathy demonstrated in my study, occipital cerebrovascular reactivity may not reflect the overall CAA burden. Instead it may be related to capillary CAA, which did show a strong, consistent occipital predominance. Ponezumab is known to reduce CAA in leptomeningeal and brain vessels, but capillaries were removed from the samples as part of the processing. Its effect on capillary CAA is not known.[280]

The frequency and severity of CAA in the cerebellum is unclear, with some studies showing little involvement,[263, 266] while others showing that the cerebellum is commonly affected.[226, 265] I found that the cerebellum was frequently affected by moderate or severe meningeal CAA, but rarely had parenchymal CAA, capillary CAA or vasculopathy. This is relevant because the cerebellar cortex is often used as the reference tissue for amyloid PET studies in ICH and CAA, based on the assumption it only has non-specific tracer binding.[284] However, this may not be the case given the frequent presence of moderate or severe meningeal CAA seen in my study.

4.5.5 Clinical implications

CAA assessed in cortical biopsies using the Vonsattel scale appears to have high sensitivity for CAA-associated lobar ICH, regardless of age. The absence of amyloid-β in a cortical biopsy (Vonsattel grade 0) can, therefore, be used to rule out CAA-associated lobar ICH. The presence of complete replacement of a vessel wall with amyloid-β (Vonsattel grade ≥2) in a cortical biopsy has good specificity for CAA-associated ICH in those aged under 85 years and can be used to rule in CAA-associated ICH in this group. However,

its specificity is modest in those aged 85 years or above and may lead to false positive results. Assessment of capillary CAA in the cortex of the cerebral lobe affected by ICH has poor sensitivity for global cerebral capillary CAA due to the occipital predominance of this type of CAA.

4.5.6 Future directions

Dedica ted diagnostic test accuracy studies of CAA assessment in simulated cortical biopsies are needed to establish the true diagnostic accuracy. These studies should use tissue samples that reflect the size and shape of cortical biopsy samples obtained in clinical practice. The samples should be assessed masked to relevant features, including the rest of the brain autopsy to reduce diagnostic-review bias.[176]

Further large neuropathological studies in ICH are needed to rigorously assess the clinical, genetic and histopathological associations of CAA and non-CAA SVDs in ICH with adjustment for potential confounders, such as age, time delay to autopsy and Alzheimer's pathology. These studies should be prospective and unselective to ensure the results are representative of ICH. The pathological assessment should include both CAA and non-CAA SVDs in the different brain regions (cerebral lobes, deep grey matter, brainstem and cerebellum) to provide a comprehensive evaluation of SVDs. Particular attention should be paid to the significance of mixed CAA and non-CAA SVDs compared with pure CAA and pure non-CAA SVD, as well as the occipital predominance of capillary CAA in lobar ICH.

Section D. Diagnostic value of SVD imaging biomarkers in SVD-associated ICH

Chapter 5 Diagnostic test accuracy studies of the original and modified Boston criteria for CAA-associated ICH against a histopathological reference standard

Chapter 6 Diagnostic value of β-amyloid PET in SVD-associated ICH

Chapter 7 The Edinburgh CT and genetic criteria for lobar ICH associated with CAA: model development and diagnostic test accuracy study

Chapter 8 External validation studies of the Edinburgh CT-only and CT-APOE diagnostic models and criteria for CAA-associated lobar ICH

Chapter 9 Diagnostic test accuracy studies of the Edinburgh diagnostic criteria for CAA-associated lobar ICH against the modified Boston criteria

Chapter 5 Diagnostic test accuracy studies of the original and modified Boston criteria for CAA-associated ICH against a histopathological reference standard

5.1 Introduction

Multiple macrohaemorrhages and CMBs restricted to the lobar regions are the original imaging hallmarks of CAA,[253, 285] and form the basis of the Boston criteria for CAA (Table 1.1).[285, 286] The probable CAA category of the original Boston criteria had excellent specificity (100%, 95% CI 66-100) but limited sensitivity (45%, 95% CI 27-64) when assessed in 39 lobar ICH participants.[103] However, this study had several limitations, such as selection bias, variable index tests (CT and MRI with and without blood-sensitive sequences) and different sources of tissue sampling as the reference standard. Restricting these analyses to the 16 participants with blood-sensitive MRI improved the sensitivity of probable CAA (73%, 95% CI 39-93) with no reduction in the specificity. However, the numbers in this sensitivity analysis were small. Two studies have externally validated the original Boston criteria in the context of ICH,[95, 110] but both also had methodological limitations, such as small sample sizes, selection bias and partial verification bias, where the results of the index test influence whether the reference standard was performed.

More recently, cortical superficial siderosis detected on blood-sensitive MRI has been shown to be associated with CAA.[108, 110, 111] The inclusion of cortical superficial siderosis as a separate lobar haemorrhagic lesion in the Boston criteria (Table 1.1) resulted in a non-significant increase in the sensitivity of the probable CAA category from 90% (95% CI 76-96) to 95% (95% CI 83-99), with the specificity remaining 81% (95% CI 62-93) in the study by Linn et al.[110] The so-called “modified Boston criteria” are now the non-invasive *in vivo* reference standard for diagnosing CAA-associated

ICH[167] and are frequently used in clinical practice and in research to guide further, often invasive, investigations, treatment decisions and recruitment into studies.[167, 168] However, there were limitations in the development of the modified Boston criteria, such as selection bias, partial verification bias and no masking of the reference standard assessment. Furthermore, the modified Boston criteria have never been externally validated. Their diagnostic accuracy and their impact on patient care is therefore uncertain.

5.2 Aim

I aimed to perform separate diagnostic test accuracy studies of the original and modified Boston criteria (index tests) against a histopathological assessment of CAA (reference standard) in a cohort study of SVD-associated ICH.

5.3 Methods

5.3.1 Study design and participants

I used data from the prospective LINCHPIN study (Section 2.1.3.2). I included consecutive adult participants (aged ≥16 years) living in the NHS Lothian Health Board region who had an ICH (first-ever or recurrent) between 1st June 2010 and 31st May 2016 inclusive, and who underwent a research brain MRI and subsequently died and had a research brain autopsy (Figure 3.3).

For the primary analysis I included participants regardless of the ICH location (lobar or non-lobar) as this was the approach used by Linn et al in the development of the modified Boston criteria.[110] However, there is no statistical association between CAA and deep ICH.[153] Furthermore, the original Boston criteria were developed in patients with only lobar ICH.[103] Therefore, I performed a sensitivity analysis excluding participants with non-lobar ICH.

I excluded patients with exclusively extra-axial intracranial haemorrhage, ICH secondary to an underlying cause other than SVDs, and those without research brain MRI with T2*-weighted GRE and research brain autopsy.

5.3.2 Baseline data collection

The RUSH team collected demographics and the presence of relevant co-morbidities and medication use at the time of ICH by interviewing patients and their relatives and reviewing medical records as described in the methods chapter (Section 2.1.7).

We determined APOE genotype from peripheral blood or cerebellar tissue as described in Section 2.3.

5.3.3 Index test

Research brain MRI was performed around six months after the index ICH using standardised parameters described in Section 2.2.3.

I rated the brain MRI scans for the presence of SVD biomarkers using the standardised pro forma described within the methods chapter (Appendix 2).[77] I classified the total brain MRI SVD burden using the MRI SVD burden score[197] and the MRI CAA SVD burden score[214] as described in Section 2.2.3.3. I used the T2*-weighted GRE sequence to categorise scans as probable CAA, possible CAA or no CAA according to the original and modified Boston criteria (Table 1.1).[110, 285, 286]

I pre-specified probable CAA as the positive index test result and possible CAA or no CAA as the negative index test result because probable CAA is the key diagnostic cut-off used in clinical practice and research.[103, 110, 167] This cut-off will maximise the specificity of the criteria, and be most useful for ruling in CAA.

I performed a pre-specified sensitivity analysis using no CAA as the negative index test result and possible CAA or probable CAA as the positive index test result as this cut-off will maximise the sensitivity of the criteria, and be most useful for ruling out CAA.

I performed all assessments masked to clinical, diagnostic brain CT, genetic and histopathological data.

5.3.4 Reference standard

Research brain autopsies were performed within five days of death according to a standard operating procedure as described in the methods chapter.[217] A single neuropathologist (Professor C Smith) assessed samples for CAA severity by using a consensus scale (Table 2.6).[36] Professor Smith performed all histopathological assessments masked to clinical, imaging and genetic data.

I pre-specified the positive reference standard test result (CAA-associated ICH) as CAA severity of ≥2 on the Vonsattel scale (replacement of whole vessel wall by amyloid-β in the most severely affected vessel in any brain sample)[253] as this was the cut-off used to develop the modified Boston criteria.[110] Vonsattel grade ≥2 corresponds to ≥2 (some circumferential amyloid-β) parenchymal or meningeal CAA on the consensus scale developed by Love et al.[36]

To assess the overall severity of CAA, I separately summed the meningeal CAA and parenchymal CAA scores for all cerebral lobes from the consensus scale developed by Love et al.[36] I graded summed scores to produce separate global cerebral meningeal CAA and parenchymal CAA scores (0=none, 1-8=mild, 9-16=moderate, 17-24=severe) as described in section 4.3.3.

5.3.5 Statistical analysis

I compared the frequency of clinical and diagnostic non-contrast brain CT features, APOE genotype, and MRI SVD biomarkers in participants classified as positive on the histopathological reference standard (CAA-associated ICH) versus negative on the histopathological reference standard (not CAA-associated ICH) using χ^2 test (or Fisher's exact test, where appropriate) for categorical variables and the Mann-Whitney U test for non-normally distributed continuous variables.

I assessed the diagnostic accuracy of the original and modified Boston criteria against the histopathological reference standard separately using sensitivity, specificity, likelihood ratios, and predictive values and their 95% CI.

I performed statistical analyses using R statistical package version 3.4.4., except for the diagnostic accuracy statistics, for which I used VassarStats Clinical Calculator 1.[269]

5.3.6 Missing data

All included participants had the index test and reference standard available.

5.3.7 Sample size

I did not perform a sample size calculation. Instead, I used the largest sample possible from the on-going prospective LINCHPIN study over six years to perform a preliminary analysis.

5.4 Results

5.4.1 Participants

5.4.1.1 Flow of participants

Between 1st June 2010 and 31st May 2016 there were 612 patients with spontaneous ICH presumed related to SVDs (Figure 3.3). Three hundred and fifty consented to the LINCHPIN study, of whom 160 had a research MRI of adequate quality. I included 16 participants who died and had a research brain autopsy (Figure 5.1 and Figure 5.2).

The median time between ICH and MRI (index test) was 92 days (IQR 55-123 days, range 7-369 days) and between MRI (index test) and autopsy (reference standard) was 927 days (IQR 350-1659 days, range 28-2343 days). There were no adverse events associated with the index test.

5.4.1.2 Comparison of included versus excluded LINCHPIN participants

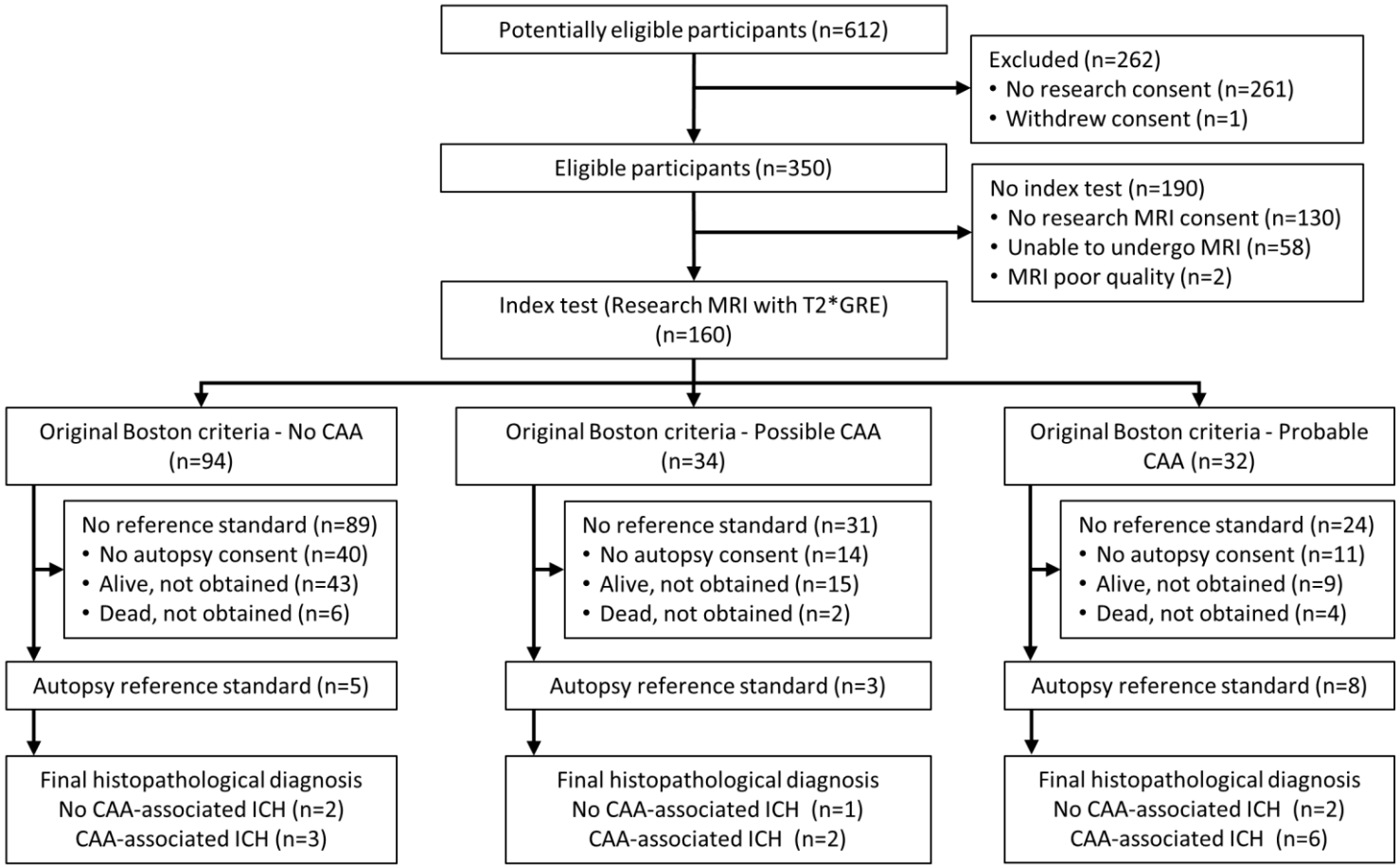
Participants included in the study were older and more likely to have a pre-ICH diagnosis of atrial fibrillation than LINCHPIN participants not included

(Table 5.1). Baseline clinical features were otherwise similar. Included participants tended to have a smaller ICH volume, less frequent intraventricular haemorrhage and finger-like projections and less severe CT SVD score (Table 5.2). However, these differences were not statistically significant.

5.4.1.3 Comparison of participants with the index test who underwent the reference standard versus those who did not

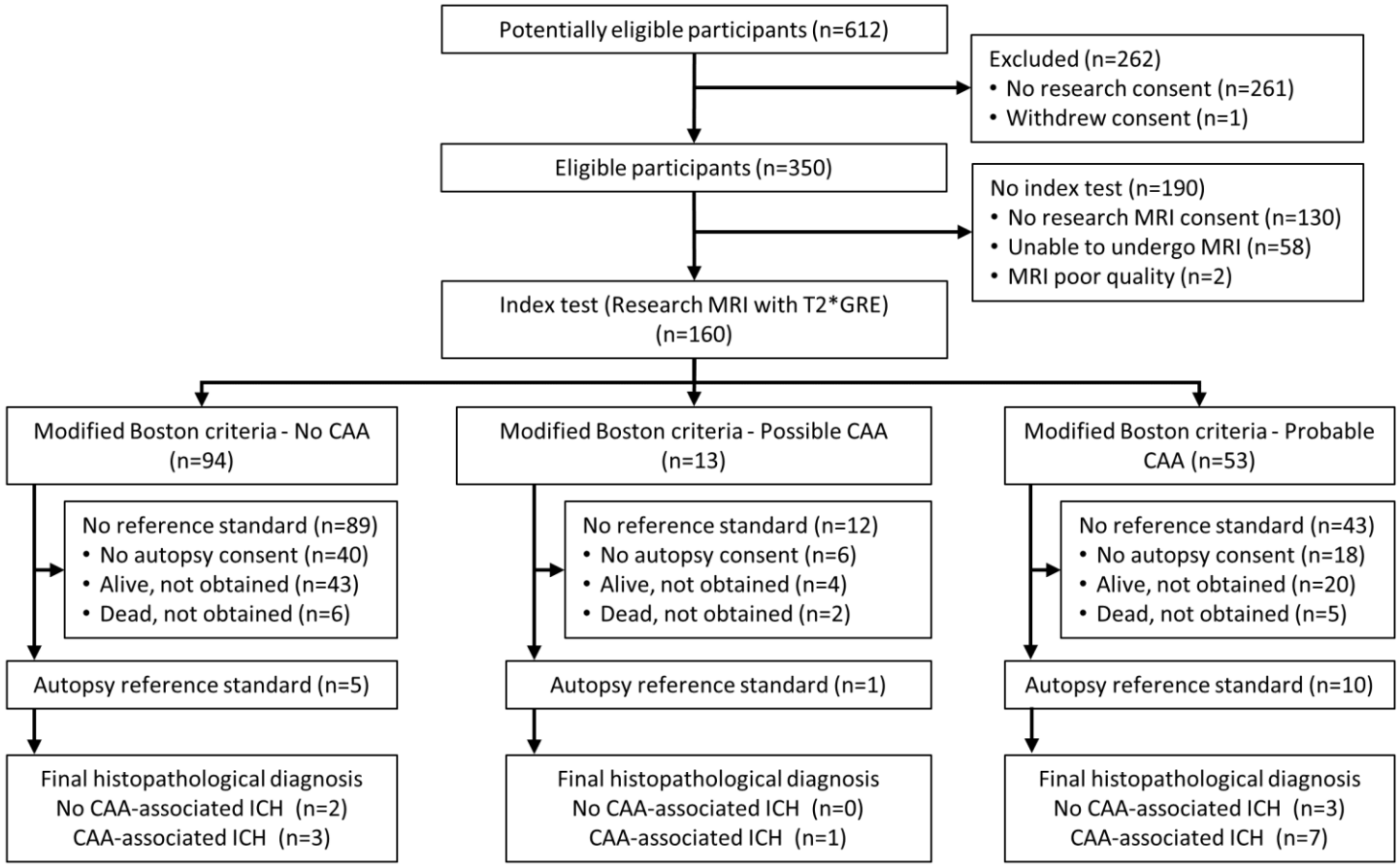
Participants who underwent both the index test (MRI) and reference standard (autopsy) were older with more frequent pre-ICH dementia and atrial fibrillation, a higher median pre-ICH modified Rankin scale score, more severe CT SVD score and more advanced atrophy compared with those who had the index test alone (Table 5.3, Table 5.4 and Table 5.5). The frequency of probable CAA according to both the original and modified Boston criteria was higher in those with both the index test and reference standard compared with those only undergoing the index test.

Figure 5.1 Flow of participants in the original Boston criteria versus autopsy reference standard LINCHPIN DTA study



CAA = cerebral amyloid angiopathy. DTA = diagnostic test accuracy. GRE = gradient recalled echo. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. MRI = magnetic resonance imaging.

Figure 5.2 Flow of participants through the modified Boston criteria versus autopsy reference standard LINCHPIN DTA study



CAA = cerebral amyloid angiopathy. DTA = diagnostic test accuracy. GRE = gradient recalled echo. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. MRI = magnetic resonance imaging

Table 5.1 Baseline clinical characteristics in LINCHPIN participants with both MRI index test and histopathological reference standard (included in the study) versus those without (not included in the study).

	Not included in study (n=335)		Included in study (n=16)		p value
Age (years); median (IQR)	76	(66-83)	82	(79-83)	0.020
Sex					
Female	167	(50)	9	(56)	0.617
Male	168	(50)	7	(44)	
Co-morbidities					
Hypertension	218	(65)	11	(69)	0.763
Ischaemic stroke*	41	(12)	3	(19)	0.435
Transient ischaemic attack*	39	(12)	1	(6)	1.000
Dementia*	32	(10)	3	(19)	0.206
Diabetes*	37	(11)	0	(0)	0.393
Atrial fibrillation*	58	(17)	8	(50)	0.004
Myocardial infarction*	22	(7)	2	(13)	0.300
Hyperlipidaemia*	44	(13)	3	(19)	0.459
Smoking status*					
Current	62	(19)	2	(13)	
Ex-smoker	109	(33)	7	(44)	0.701
Never	164	(49)	7	(44)	
Pre-ICH modified Rankin scale; median (IQR)	2	(1-3)	2	(1-3)	0.526
Medications on admission					
Antiplatelet drug(s)	121	(36)	5	(31)	0.691
Anticoagulant drug(s)*	47	(14)	4	(25)	0.266
Antihypertensive drug(s)	159	(48)	8	(50)	0.843
Admission GCS; median (IQR)	14	(11-15)	14	(14-15)	0.207

Data are n (%) or median (IQR). * Fisher's exact test. GCS = Glasgow coma scale. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. MRI = magnetic resonance imaging. SVD = small vessel disease.

Table 5.2 ICH location and non-contrast diagnostic brain CT characteristics in LINCHPIN participants with both MRI index test and histopathological reference standard (included in the study) versus those without (not included in the study).

	Not included in study (n=335)	**Included in study (n=16)**	**p value**
ICH location			
Lobar	163 (49)	11 (69)	
Deep	138 (41)	3 (19)	0.174
Infratentorial	34 (10)	2 (13)	
ICH volume; median (IQR)	16 (5-41)	10 (4-18)	0.095
Intraventricular haemorrhage	132 (39)	3 (19)	0.097
Subarachnoid haemorrhage	133 (40)	8 (50)	0.412
Subdural haemorrhage*	30 (9)	0 (0)	0.379
Finger-like projections	48 (15)	0 (0)	0.141
CT SVD score			
0	122 (37)	7 (44)	
1	121 (37)	8 (50)	0.075
2	73 (22)	0 (0)	
3	11 (3)	1 (6)	

Data are n (%) or median (IQR). * Fisher's exact test. CT = computed tomography. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. MRI = magnetic resonance imaging. SVD = small vessel disease.

Table 5.3 Baseline clinical features in LINCHPIN participants with research MRI who had a research autopsy versus those who did not

	Index test only (n=144)		Index test and reference standard (n=16)		p value
Age (years); median (IQR)	68	(56-76)	82	(79-83)	<0.001
Sex					
Female	66	(46)	9	(56)	0.428
Male	78	(54)	7	(44)	
Co-morbidities					
Hypertension	85	(59)	11	(69)	0.451
Ischaemic stroke*	13	(9)	3	(19)	0.203
Transient ischaemic attack*	9	(6)	1	(6)	1.000
Dementia*	0	(0)	3	(19)	<0.001
Diabetes*	11	(8)	0	(0)	0.604
Atrial fibrillation*	11	(8)	8	(50)	<0.001
Myocardial infarction*	4	(3)	2	(13)	0.111
Hyperlipidaemia*	20	(14)	3	(19)	0.705
Smoking status*					
Current	28	(19)	2	(13)	
Ex-smoker	41	(29)	7	(44)	0.528
Never	75	(52)	7	(44)	
Pre-ICH modified Rankin scale; median (IQR)	1	(1-1)	2	(1-3)	0.010
Medications on admission					
Antiplatelet drug(s)*	42	(29)	5	(31)	1.000
Anticoagulant drug(s)*	12	(8)	4	(25)	0.058
Antihypertensive drug(s)	57	(40)	8	(50)	0.421
Admission GCS; median (IQR)	15	(15-15)	14	(14-15)	0.161

Data are n (%) or median (IQR). * Fisher's exact test. GCS = Glasgow coma scale. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. MRI = magnetic resonance imaging. SVD = small vessel disease.

Table 5.4 ICH location and non-contrast diagnostic brain CT characteristics in LINCHPIN participants with research MRI who had a research autopsy versus those who did not

	Index test only (n=144)	Index test and reference standard (n=16)	p value
ICH location*			
Lobar	66 (46)	11 (69)	
Deep	59 (41)	3 (19)	0.170
Infratentorial	19 (13)	2 (13)	
ICH volume; median (IQR)	9 (3-22)	10 (4-18)	0.903
Intraventricular haemorrhage*	31 (22)	3 (19)	1.000
Subarachnoid haemorrhage	47 (33)	8 (50)	0.165
Subdural haemorrhage*	10 (7)	0 (0)	0.600
Finger-like projections*	15 (11)	0 (0)	0.370
CT SVD score			
0	78 (57)	7 (44)	
1	34 (25)	8 (50)	0.022
2	24 (18)	0 (0)	
3	1 (1)	1 (6)	

Data are n (%) or median (IQR). * Fisher's exact test. CT = computed tomography. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. MRI = magnetic resonance imaging. SVD = small vessel disease.

Table 5.5 Research brain MRI features in LINCHPIN participants with research MRI who had a research autopsy versus those who did not

	Index test only (n=144)		Index test and reference standard (n=16)		p value
Periventricular Fazekas score; median (IQR)	3	(2-4)	4	(3-4)	0.076
Deep Fazekas score; median (IQR)	3	(2-3)	3	(2-3)	0.936
Central atrophy; median (IQR)	3	(1-4)	3	(3-4)	0.034
Cortical atrophy; median (IQR)	2	(1-3)	3	(2-5)	0.008
Basal ganglia PVS; median (IQR)	1	(1-2)	2	(1-2)	0.427
Centrum semiovale PVS; median (IQR)	3	(2-4)	3	(2-3)	0.551
Multiple ICH*	21	(15)	45	(31)	0.142
Cortical superficial siderosis	56	(39)	10	(63)	0.069
Any lobar CMB	48	(33)	7	(44)	0.405
Any deep CMB*	38	(26)	3	(19)	0.763
Any cerebellar CMB*	15	(10)	3	(19)	0.395
Any brainstem CMB*	11	(8)	2	(13)	0.622
Any CMB	67	(47)	8	(50)	0.792
MRI SVD burden score; median (IQR)	3	(2-4)	2	(2-4)	0.575
MRI CAA SVD burden score; median (IQR)	2	(2-3)	3	(2-4)	0.149
Original Boston Criteria*					
No CAA	89	(62)	5	(31)	
Possible CAA	31	(22)	4	(25)	0.020
Probable CAA	24	(17)	7	(44)	
Modified Boston Criteria*					
No CAA	89	(62)	5	(31)	
Possible CAA	12	(8)	1	(6)	0.034
Probable CAA	43	(30)	10	(63)	

Data are n (%) or median (IQR). * Fisher's exact test. CAA = cerebral amyloid angiopathy. CMB = cerebral microbleed. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. MRI = magnetic resonance imaging. PVS = perivascular space. SVD = small vessel disease.

5.4.1.4 Baseline clinical characteristics associated with the presence and absence of CAA on histopathology

Eleven participants were classified as CAA-associated ICH by the reference standard and five as not CAA-associated ICH. There were no significant differences in demographics, co-morbidities or non-contrast diagnostic CT brain features between the CAA-associated ICH group compared with the non-CAA associated ICH group (Table 5.6 and Table 5.7). APOE ε2 and ε4 allele possession occurred exclusively in the CAA-associated ICH, however these differences were only statistically significant for APOE ε4. The time between ICH and MRI was significantly shorter for CAA-associated ICH (median 88 days, IQR 54-97) versus the non-CAA associated ICH group (median 123 days, IQR 123-129). There was no significant difference in the number of days between MRI and autopsy between CAA-associated and non-CAA-associated ICH groups (Table 5.6).

Participants included in this study tend to be older than those included in the original[103] and modified[110] Boston criteria studies (Table 5.8).

Table 5.6 Baseline clinical characteristics in DTA study participants classified as CAA-associated versus non-CAA-associated ICH by the histopathological reference standard

	ICH not CAA-associated (n=5)		CAA-associated ICH (n=11)		p value
Age (years); median (IQR)	82	(79-83)	81	(80-83)	0.787
Sex*					
Female	3	(60)	6	(55)	1.000
Male	2	(40)	5	(45)	
Co-morbidities					
Hypertension*	5	(100)	6	(55)	0.119
Ischaemic stroke*	0	(0)	3	(27)	0.509
Transient ischaemic attack*	0	(0)	1	(9)	1.000
Dementia*	0	(0)	3	(27)	0.509
Diabetes	0	(0)	0	(0)	NA
Atrial fibrillation*	4	(80)	4	(36)	0.282
Myocardial infarction*	2	(40)	0	(0)	0.083
Hyperlipidaemia*	1	(20)	2	(18)	1.000
Smoking status*					
Current	1	(20)	1	(9)	
Ex-smoker	3	(60)	4	(36)	0.461
Never	1	(20)	6	(55)	
Pre-ICH modified Rankin scale; median (IQR)	2	(1-3)	1	(1-3)	0.668
APOE ε2 possession*	0	(0)	6	(55)	0.093
APOE ε4 possession*	0	(0)	7	(64)	0.033
Medications on admission					
Antiplatelet drug(s)*	3	(60)	2	(18)	0.245
Anticoagulant drug(s)*	1	(20)	3	(27)	1.000
Antihypertensive drug(s)*	3	(60)	5	(45)	1.000
Admission GCS; median (IQR)	14	(14-15)	14	(13-15)	0.715
ICH type*					
First-ever	5	(100)	9	(82)	1.000
Recurrent	0	(0)	2	(18)	
Time between index ICH and MRI (days); median (IQR)	123	(123-129)	88	(54-97)	0.042
Time between MRI and autopsy (days); median (IQR)	496	(357-1789)	993	(398-1482)	0.872

Data are n (%) or median (IQR). * Fisher's exact test. CAA = cerebral amyloid angiopathy. DTA = diagnostic test accuracy. GCS = Glasgow coma scale. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. MRI = magnetic resonance imaging. SVD = small vessel disease.

Table 5.7 ICH location and non-contrast diagnostic brain CT features in DTA study participants classified as CAA-associated versus non-CAA-associated ICH by the histopathological reference standard

	ICH not CAA-associated (n=5)	CAA-associated ICH (n=11)	p value
ICH location*			
Lobar	2 (40)	9 (82)	
Deep	1 (20)	2 (18)	0.101
Infratentorial	2 (40)	0 (0)	
ICH volume; median (IQR)	5 (3-11)	14 (5-19)	0.160
Intraventricular haemorrhage*	2 (40)	1 (9)	0.214
Subarachnoid haemorrhage*	2 (40)	6 (55)	1.000
Subdural haemorrhage	0 (0)	0 (0)	NA
Finger-like projections	0 (0)	0 (0)	NA
CT SVD score*			
0	4 (80)	3 (27)	
1	1 (20)	7 (64)	0.170
2	0 (0)	0 (0)	
3	0 (0)	1 (9)	

Data are n (%) or median (IQR). * Fisher's exact test. CAA = cerebral amyloid angiopathy. CT = computed tomography. DTA = diagnostic test accuracy. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. SVD = small vessel disease.

Table 5.8 Baseline characteristics of participants included in the development studies of the original and modified Boston criteria

	Original Boston criteria study†		**Modified Boston criteria study**	
	No CAA (n=10)	CAA (n=29)	No CAA (n=22)	CAA (n=38)
Age; mean (SD)	71 (6)	74 (9)	54 (18)	70 (6)
Sex				
Female	5 (50)	21 (72)	10 (45)	22 (58)
Male	5 (50)	8 (28)	12 (55)	16 (42)
Co-morbidities				
Hypertension	7 (70)	16 (57)	? (-)	? (-)
Dementia	2 (20)	7 (25)	? (-)	? (-)
Medications on admission				
Antiplatelet drug	3 (30)	6 (21)	8 (36)	10 (26)
Warfarin	6 (60)	9 (31)	3 (14)	2 (5)

Data are n (%) or mean (SD).? = not reported; † Includes participants undergoing CT, MRI without blood-sensitive imaging or MRI with blood-sensitive imaging as the index test. CAA = cerebral amyloid angiopathy. CT = computed tomography. MRI = magnetic resonance imaging.

5.4.1.5 MRI characteristics associated with CAA-associated and non-CAA-associated ICH on histopathology

Nine CAA-associated ICH cases had lobar ICH and two had a deep ICH (Table 5.9). There was no statistically significant difference in the number of ICHs, the presence of cortical superficial siderosis or the presence or number of lobar or deep CMB between CAA-associated and non-CAA-associated ICH. The severity of WMH, atrophy and PVS was similar between the groups. The median CAA SVD burden score was higher in CAA-associated ICH, although this did not reach statistical significance.

Table 5.9 MRI features (index test) in DTA study participants classified as CAA-associated versus non-CAA-associated ICH by the histopathological reference standard

	ICH not CAA-associated (n=5)		CAA-associated ICH (n=11)		p value
Multiple ICH*	1	(20)	4	(36)	1.000
ICH locations*					
Lobar	2	(40)	9	(82)	
Deep	1	(20)	2	(18)	0.101
Infratentorial	2	(40)	0	(0)	
Lobar CMB presence*	1	(20)	6	(55)	0.308
Lobar CMBs; median (IQR)	0	(0-0)	1	(0-2)	0.259
Deep CMB presence*	1	(20)	2	(18)	1.000
Deep CMBs; median (IQR)	0	(0-0)	0	(0-0)	0.803
Cerebellar CMB presence*	2	(40)	1	(9)	0.214
Cerebellar CMBs; median (IQR)	0	(0-2)	0	(0-0)	0.158
Brainstem CMB presence*	1	(20)	1	(9)	1.000
Brainstem CMBs; median (IQR)	0	(0-0)	0	(0-0)	0.491
Total CMB presence*	2	(40)	6	(55)	1.000
Total CMBs; median (IQR)	0	(0-2)	1	(0-6)	0.809
Cortical superficial siderosis*	3	(60)	7	(64)	1.000
Cortical superficial siderosis extent*					
Focal	2	(67)	1	(14)	0.183
Diffuse	1	(33)	6	(86)	
Cortical superficial siderosis location*					
Adjacent to ICH	2	(67)	1	(14)	
Distant to ICH	0	(0)	0	(0)	0.183
Both	1	(33)	6	(86)	
Periventricular Fazekas score; median (IQR)	3	(3-4)	4	(3-4)	0.273
Deep Fazekas score; median (IQR)	2	(2-3)	3	(2-3)	0.711
Central atrophy; median (IQR)	3	(2-4)	3	(3-5)	0.769
Cortical atrophy; median (IQR)	4	(2-5)	3	(2-4)	0.729
Basal ganglia PVS*					
1	2	(40)	6	(55)	
2	3	(60)	2	(18)	0.535
3	0	(0)	1	(9)	
4	0	(0)	2	(18)	
Severe basal ganglia PVS*	0	(0)	3	(27)	0.509
Centrum semiovale PVS*					
0	0	(0)	0	(0)	
1	3	(60)	3	(27)	
2	2	(40)	6	(55)	0.807
3	0	(0)	1	(9)	
4	0	(0)	1	(9)	

	ICH not CAA-associated (n=5)	CAA-associated ICH (n=11)	p value
Severe centrum semiovale PVS*	0 (0)	2 (18)	1.000
MRI SVD burden score; median (IQR)	2 (1-4)	2 (2-4)	0.507
MRI CAA SVD burden score; median (IQR)	2 (1-3)	3 (3-5)	0.072
Original Boston Criteria*			
No CAA	2 (40)	3 (27)	
Possible CAA	1 (20)	2 (18)	1.000
Probable CAA	2 (40)	6 (55)	
Modified Boston Criteria*			
No CAA	2 (40)	3 (27)	
Possible CAA	0 (0)	1 (9)	1.000
Probable CAA	3 (60)	7 (64)	

Data are n (%) or median (IQR). * Fisher's exact test. CAA = cerebral amyloid angiopathy. CMB = cerebral microbleed. DTA = diagnostic test accuracy. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. MRI = magnetic resonance imaging. MRI = magnetic resonance imaging. PVS = perivascular space. SVD = small vessel disease.

5.4.1.6 Histopathological SVDs severity in CAA-associated and non-CAA-associated ICH

Ten participants with CAA-associated lobar ICH had moderate or severe global cerebral meningeal CAA on the Love et al. consensus scale, while the other participant had mild meningeal CAA. Nine participants also had moderate or severe global cerebral parenchymal CAA, while one participant had mild parenchymal CAA and in the other participant there was no parenchymal CAA. Eight participants had associated vasculopathic changes. Seven participants had moderate or severe non-CAA SVD while the remaining four participants had mild non-CAA SVD.

All five participants with non-CAA-associated ICH had absent global cerebral parenchymal CAA. There was mild global cerebral meningeal CAA in two participants with non-CAA-associated ICH, while in the rest there was no meningeal CAA. None of them had vasculopathy. Four of the non-CAA-associated ICH participants had severe non-CAA SVD, while the other participants had moderate non-CAA SVD.

5.4.2 Index test versus reference standard

5.4.2.1 Original Boston criteria

Table 5.10 shows the cross-tabulation of the original Boston criteria (index test) against the histopathological assessment for CAA (reference standard). Six out of the 11 CAA-associated ICH cases were classified as probable CAA by the original Boston criteria, resulting in a sensitivity of 55% (95% CI 25-82, Table 5.11). Three out of the five non-CAA-associated ICH controls were classified as either no CAA or possible CAA, resulting in a specificity of 60% (95% CI 17-93).

Examples of true positive (Figure 5.3 and Figure 5.4) and true negative (Figure 5.5 and Figure 5.6) cases are shown below. The clinical and imaging features for the two false positive and five false negative cases using the original Boston criteria are summarised in Figure 5.7 and Figure 5.8 respectively.

In a sensitivity analysis using the no CAA category as the negative index test cut-off, the sensitivity increased to 73% (95% CI 39-93), with a decrease in the specificity to 40% (95% CI 7-83).

Table 5.10 Cross-tabulations of the original Boston criteria classifications using probable CAA or possible/probable CAA as the index test cut off against the histopathological reference standard

Original Boston criteria (Index test)	**Vonsattel ≥2 (Reference standard)**		
	No CAA	CAA	Total
No/possible CAA	3	5	8
Probable CAA	2	6	8
Total	5	11	16
No CAA	2	3	5
Possible/probable CAA	3	8	11
Total	5	11	16

CAA = cerebral amyloid angiopathy. DTA = diagnostic test accuracy. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome.

Table 5.11 DTA statistics for the original Boston criteria CAA using probable CAA or possible/probable CAA as the index test cut off

	Original Boston criteria	
	Probable CAA	Possible/probable CAA
Sensitivity	55 (25-82)	73 (39-93)
Specificity	60 (17-93)	40 (7-83)
Positive likelihood ratio	1.4 (0.4-4.5)	1.2 (0.5-2.7)
Negative likelihood ratio	0.8 (0.3-1.9)	0.7 (0.1-3.2)
Positive predictive value	75 (36-96)	73 (39-93)
Negative predictive value	38 (10-74)	40 (7-83)

Data are percentage or ratio (95% CI). CAA = cerebral amyloid angiopathy. DTA = diagnostic test accuracy. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome.

Figure 5.3 True positive result for the original and modified Boston criteria against the histopathological reference standard.

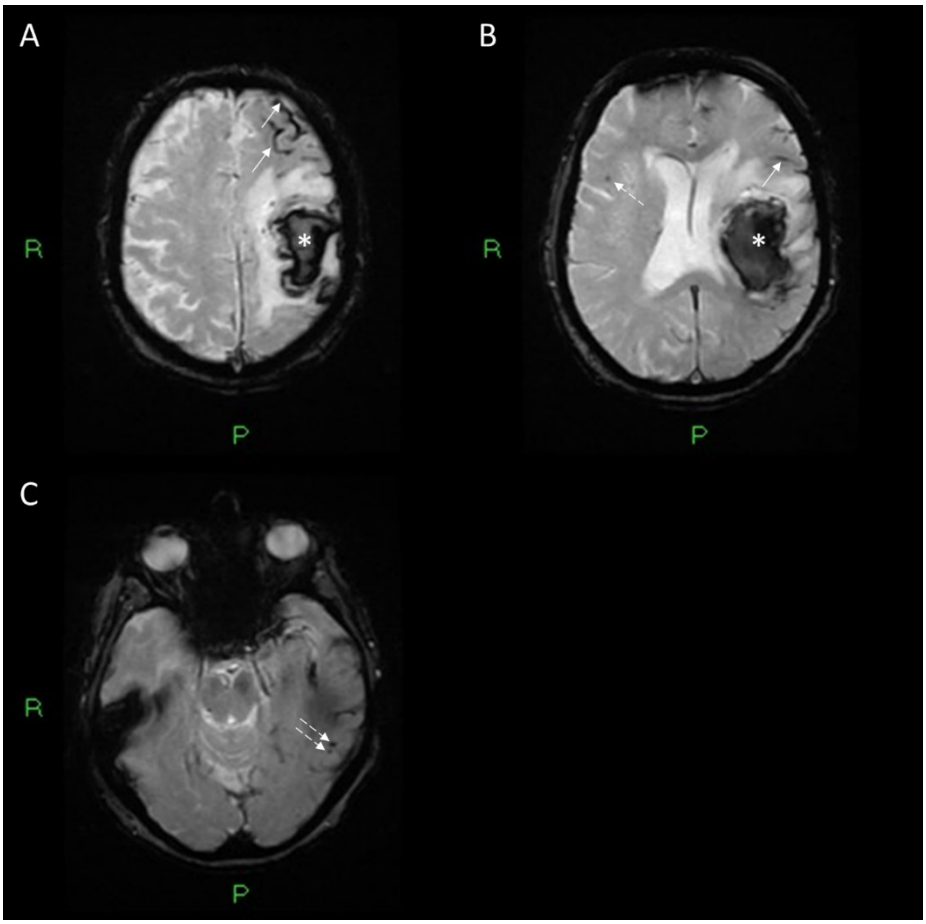
Axial T2*-weighted GRE images showing

A. Lobar ICH with its epicentre in the left frontal lobe (asterisk) and left frontal superficial siderosis (white arrows).

B. Lobar ICH with its epicentre in the left frontal lobe (asterisk), left frontal superficial siderosis (white arrow) and right frontal lobar cerebral microbleed (dotted arrow).

C. Two lobar cerebral microbleeds in the left temporal lobe (dotted arrows).

The participant is classified as probable CAA by both the original Boston criteria (lobar ICH and lobar cerebral microbleeds) and the modified Boston criteria (lobar ICH, lobar cerebral microbleeds and cortical superficial siderosis). Histopathological assessment showed a CAA-associated ICH (grade 3 CAA on the Vonsattel scale). There was severe parenchymal and meningeal global cerebral CAA with vasculopathy on the Love et al scale. Mild non-CAA SVD was present.



CAA = cerebral amyloid angiopathy. GRE = gradient recalled echo. ICH = intracerebral haemorrhage. MRI = magnetic resonance imaging. P = posterior. R = right. SVD = small vessel disease.

Figure 5.4 True positive result for the original and modified Boston criteria against the autopsy histopathological reference standard

Axial T2*-weighted GRE images showing

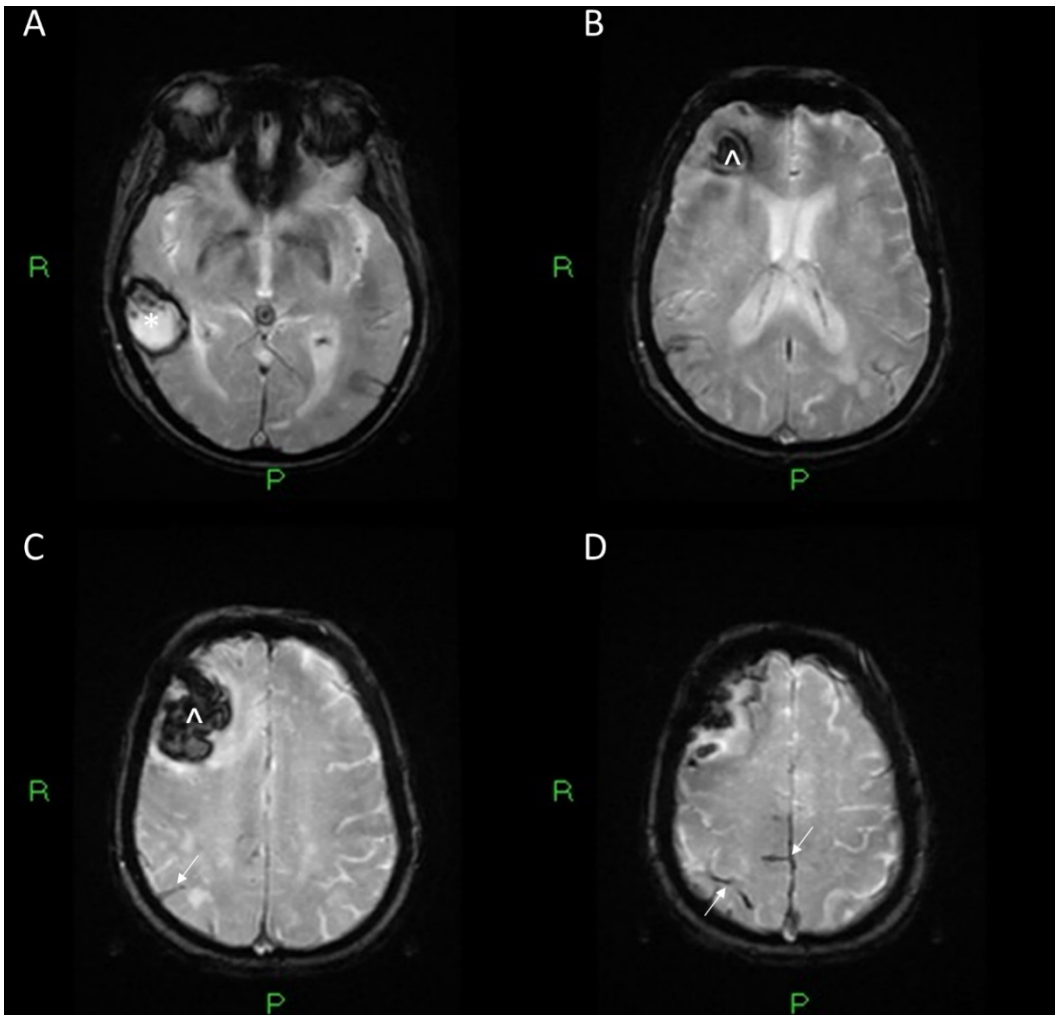
A. Lobar ICH with its epicentre in the right temporal lobe (asterisk).

B. Second lobar ICH with its epicentre in the right frontal lobe (chevron).

C. Second lobar ICH centred in the right frontal lobe (chevron) and right parietal cortical superficial siderosis (white arrow).

D. Right parietal cortical superficial siderosis (white arrows).

The participant is classified as probable CAA by both the original Boston criteria (two lobar ICHs) and the modified Boston criteria (two lobar ICHs and cortical superficial siderosis). Histopathology showed a CAA-associated ICH (grade 3 CAA on the Vonsattel scale). There was severe parenchymal and meningeal global cerebral CAA with vasculopathy on to the Love et al scale. Moderate non-CAA SVD was present.



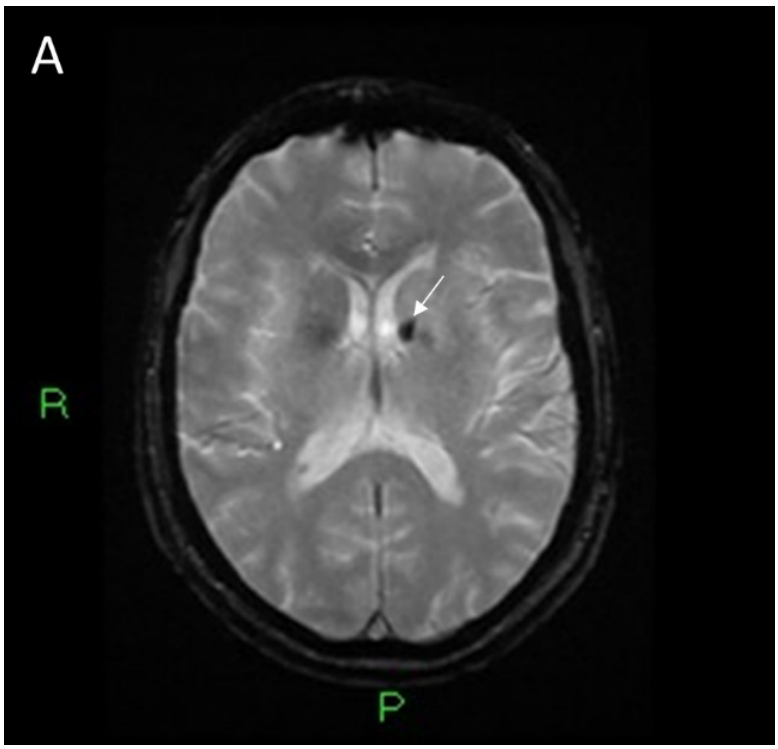
CAA = cerebral amyloid angiopathy. GRE = gradient recalled echo. ICH = intracerebral haemorrhage. MRI = magnetic resonance imaging. P = posterior. R = right. SVD = small vessel disease.

Figure 5.5 True negative result for the original and modified Boston criteria against the autopsy histopathological reference standard

Axial T2*-weighted GRE image showing

A. Deep ICH with its epicentre in the left caudate head nucleus (white arrow).

The participant is classified as no CAA by both the original and modified Boston criteria (single deep ICH). Histopathological assessment showed a non-CAA-associated ICH (grade 0 CAA on the Vonsattel scale). There was absent parenchymal and meningeal global cerebral CAA and no vasculopathy on to the Love et al scale. Severe non-CAA SVD was present.



CAA = cerebral amyloid angiopathy. GRE = gradient recalled echo. ICH = intracerebral haemorrhage. MRI = magnetic resonance imaging. P = posterior. R = right. SVD = small vessel disease.

Figure 5.6 True negative test result for the original and modified Boston criteria against the autopsy histopathological reference standard

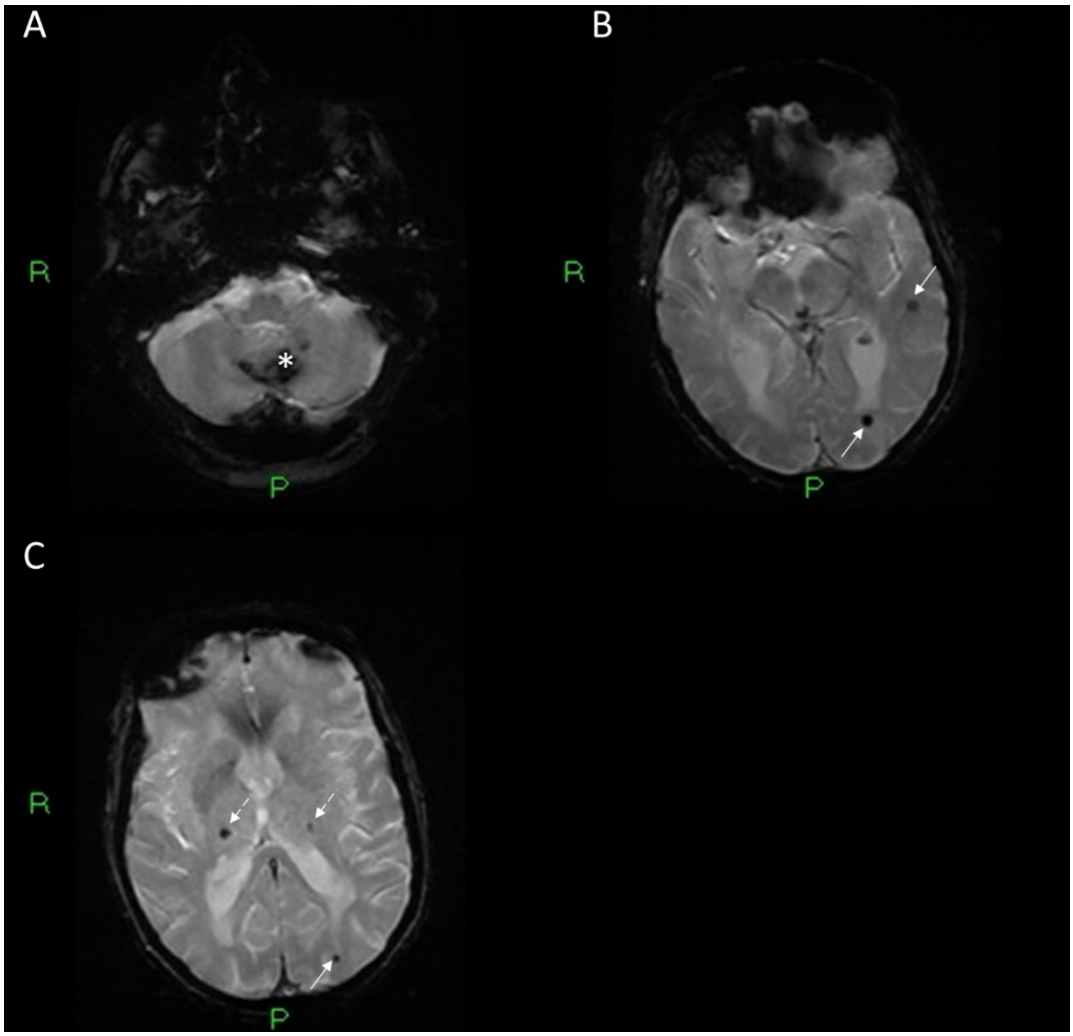
Axial T2*-weighted GRE images showing

A. Lobar ICH with its epicentre in the left cerebellar hemisphere (asterisk).

B. Lobar cerebral microbleeds in the left occipital and temporal lobes (white arrows).

C. Lobar cerebral microbleed in the left occipital lobe (white arrow) and deep cerebral microbleeds in the thalami (white dotted arrows).

The participant is classified as no CAA by both the original and modified Boston criteria (lobar haemorrhage and mixed lobar and deep cerebral microbleeds). Histopathological assessment showed a non-CAA-associated ICH (grade 0 CAA on the Vonsattel scale). There was absent parenchymal and meningeal global cerebral CAA and no vasculopathy according to the Love et al scale. Severe non-CAA SVD was present.

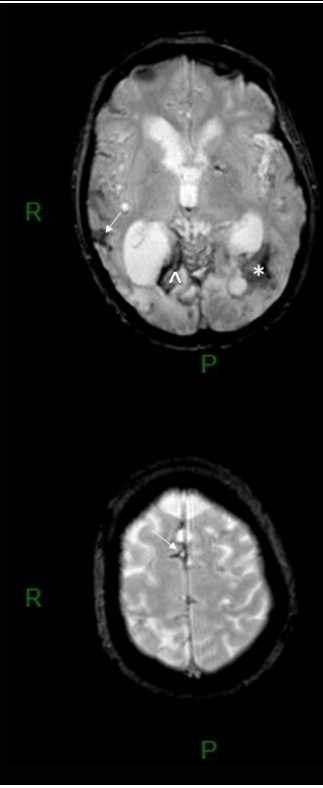


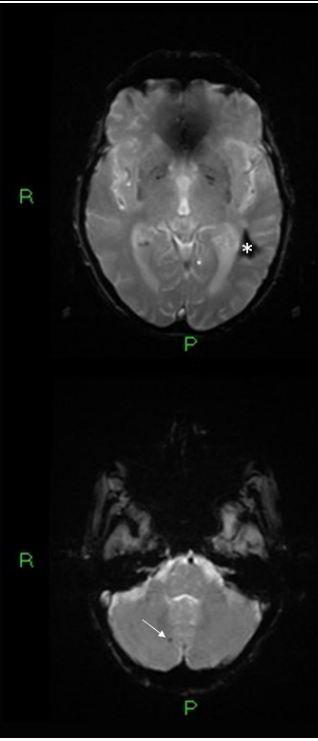
CAA = cerebral amyloid angiopathy. GRE = gradient recalled echo. ICH = intracerebral haemorrhage. MRI = magnetic resonance imaging. P = posterior. R = right. SVD = small vessel disease.

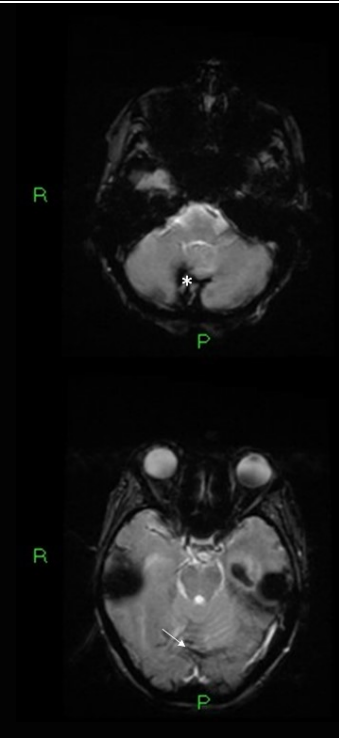
Figure 5.7 Discrepancies between the original and modified Boston criteria and the autopsy reference standard using probable CAA as the positive index test result

A and B False positive index test result for both the original and modified Boston criteria

C False positive index test result for the modified Boston criteria

	Clinical details	MRI features	Boston criteria classification	Example T2*-weighted GRE images	Histopathology features
A	82 year-old female First-ever symptomatic ICH Pre-ICH history of hypertension No pre-ICH antiplatelet or anticoagulant drug use APOE genotype ε3/3	Two lobar ICHs (left temporal lobe [asterisk] and right parietal lobe [chevron]) No microbleeds Diffuse cortical superficial siderosis (solid arrows)	Original – Probable CAA Modified – Probable CAA		Vonsattel grade 1 (non CAA-associated ICH) Mild meningeal global cerebral CAA Absent parenchymal global cerebral CAA No vasculopathy Moderate non-CAA SVD Time from MRI to autopsy = 89 days

	Clinical details	MRI features	Boston criteria classification	Example T2*-weighted GRE images	Histopathology features
B	83 year-old female First-ever symptomatic ICH Pre-ICH history of hypertension Pre-ICH antiplatelet drug use APOE genotype ε3/3	Left temporal lobar ICH (asterisk) Cerebellar microbleeds (solid arrow) No cortical superficial siderosis	Original – Probable CAA Modified – Probable CAA		Vonsattel grade 1 (non CAA-associated ICH) Mild meningeal global cerebral CAA Absent parenchymal global cerebral CAA No vasculopathy Severe non-CAA SVD Time from MRI to autopsy = 496 days

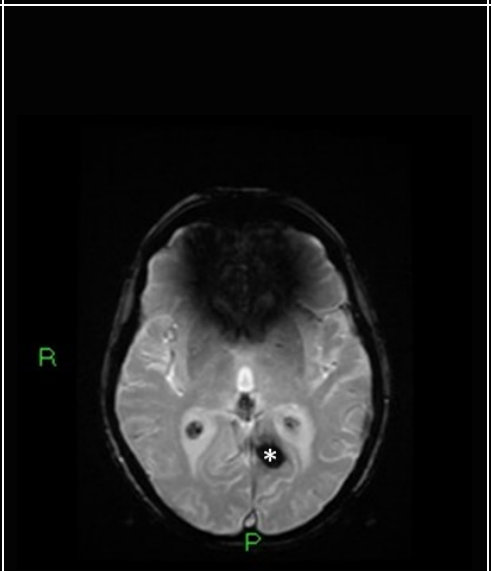
	Clinical details	MRI features	Boston criteria classification	Example T2*-weighted GRE images	Histopathology features
C	79 year-old female First-ever symptomatic ICH Pre-ICH history of hypertension Pre-ICH antiplatelet drug use APOE genotype ε3/3	Right cerebellar hemisphere lobar ICH (asterisk) No microbleeds Focal cortical superficial siderosis (solid arrow)	Original – Possible CAA Modified – Probable CAA		Vonsattel grade 1 (non CAA-associated ICH) Mild meningeal global cerebral CAA Absent parenchymal global cerebral CAA No vasculopathy Severe non-CAA SVD Time from MRI to autopsy = 2343 days

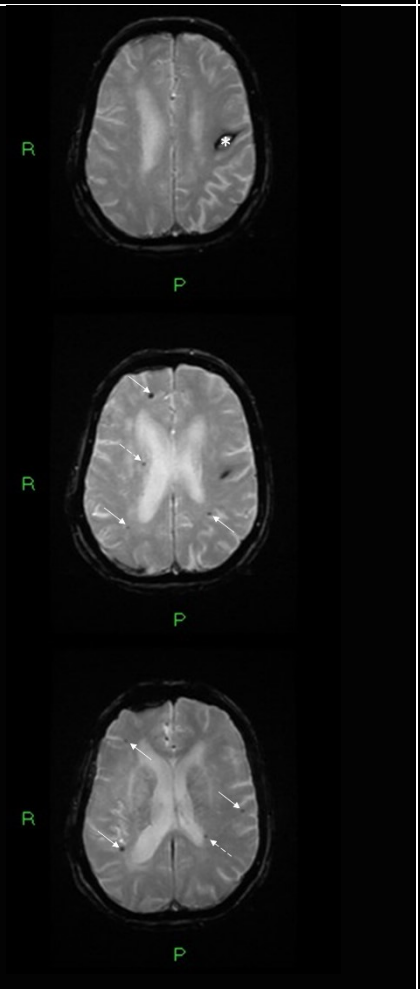
APOE = apolipoprotein E. CAA = cerebral amyloid angiopathy. GRE = gradient recalled echo. ICH = intracerebral haemorrhage. MRI = magnetic resonance imaging. SVD = small vessel disease.

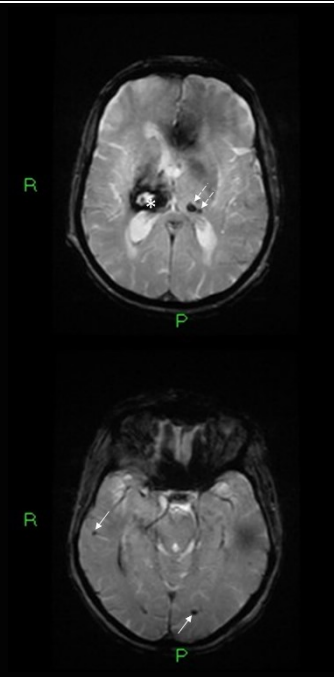
Figure 5.8 Discrepancies between the original and modified Boston criteria and the autopsy reference standard using probable CAA as the positive index test result

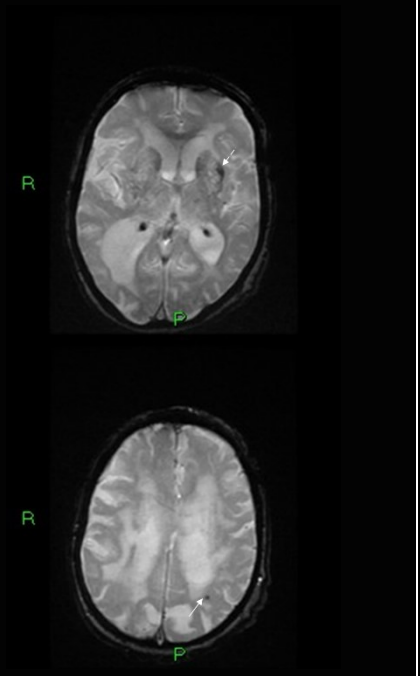
A - D False negative index test result for both the original and modified Boston criteria

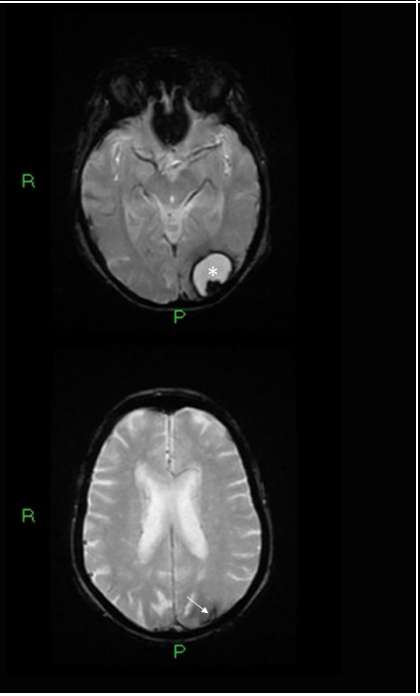
E False positive index test result for the original Boston criteria

	Clinical details	MRI features	Boston criteria classification	Example T2*-weighted GRE images	Histopathology features
A	88 year-old female First-ever symptomatic ICH No pre-ICH history of hypertension No pre-ICH antiplatelet or anticoagulant drug use APOE genotype ε2/3	Left parietal lobar ICH (asterisk) No microbleeds No cortical superficial siderosis	Original – Possible CAA Modified – Possible CAA		Vonsattel grade 3 (CAA-associated ICH) Severe meningeal global cerebral CAA Severe parenchymal global cerebral CAA Vasculopathy present Severe non-CAA SVD Time from MRI to autopsy = 1897 days

	Clinical details	MRI features	Boston criteria classification	Example T2*-weighted GRE images	Histopathology features
B	83 year-old female First-ever symptomatic ICH No pre-ICH history of hypertension Pre-ICH antiplatelet drug use APOE genotype ε3/4	Left frontal lobar ICH (asterisk) Lobar microbleeds (solid arrows) Deep microbleeds in the periventricular white matter (dashed arrow) No cortical superficial siderosis	Original – No CAA Modified – No CAA		Vonsattel grade 3 (CAA-associated ICH) Severe meningeal global cerebral CAA Severe parenchymal global cerebral CAA No vasculopathy Severe non-CAA SVD Time from MRI to autopsy = 266 days

	Clinical details	MRI features	Boston criteria classification	Example T2*-weighted GRE images	Histopathology features
C	81 year-old female First-ever symptomatic ICH Pre-ICH history of hypertension Pre-ICH anticoagulant drug use (Admission INR 2.4) APOE genotype ε2/4	Right thalamic ICH (asterisk) Lobar microbleeds (solid arrows) Deep microbleeds (dashed arrows) No cortical superficial siderosis	Original – No CAA Modified – No CAA		Vonsattel grade 2 (CAA-associated ICH) Mild meningeal global cerebral CAA Absent parenchymal global cerebral CAA No vasculopathy Severe non-CAA SVD Time from MRI to autopsy = 1263 days

	Clinical details	MRI features	Boston criteria classification	Example T2*-weighted GRE images	Histopathology features
D	83 year-old male First-ever symptomatic ICH Pre-ICH history of hypertension Pre-ICH anticoagulant drug use (Admission INR 2.1) APOE genotype ε3/4	Left basal ganglia ICH (dashed arrow) Lobar microbleed (solid arrows) No deep microbleeds No cortical superficial siderosis	Original – No CAA Modified – No CAA		Vonsattel grade 3 (CAA-associated ICH) Moderate meningeal global cerebral CAA Moderate parenchymal global cerebral CAA Vasculopathy present Severe non-CAA SVD Time from MRI to autopsy = 357 days

	Clinical details	MRI features	Boston criteria classification	Example T2*-weighted GRE images	Histopathology features
E	90 year-old female First-ever symptomatic ICH Pre-ICH history of hypertension Pre-ICH anticoagulant drug use (Admission INR 2.3) APOE genotype ε3/3	Left occipital lobar ICH (asterisk) No microbleeds Focal cortical superficial siderosis (solid arrow)	Original – Possible CAA Modified –Probable CAA		Vonsattel grade 2 (CAA-associated ICH) Moderate meningeal global cerebral CAA Mild parenchymal global cerebral CAA No vasculopathy Severe non-CAA SVD Time from MRI to autopsy = 466 days

APOE = apolipoprotein E. CAA = cerebral amyloid angiopathy. GRE = gradient recalled echo. ICH = intracerebral haemorrhage. MRI = magnetic resonance imaging. SVD = small vessel disease.

5.4.2.2 Modified Boston criteria

The inclusion of cortical superficial siderosis as a distinct haemorrhagic lesion (modified Boston criteria –Table 1.1) resulted in the upgrading from possible CAA to probable CAA in two participants. The cross-tabulation of the modified Boston criteria against histopathology reference standard is shown in Table 5.12.

The sensitivity of the modified Boston criteria using the probable CAA category as the index test cut-off was 64% (95% CI 32-88) and its specificity 40% (95% CI 7-83, Table 5.13).

Examples of true positive (Figure 5.3 and Figure 5.4) and true negative (Figure 5.5 and Figure 5.6) cases are shown above. The clinical and imaging features for the three false positive and four false negative cases are summarised in Figure 5.7 and Figure 5.8 respectively.

Using the no CAA category as the negative index test cut-off, the sensitivity and specificity of the modified Boston criteria were the same as the original Boston criteria (sensitivity 73% (95% CI 39-93), specificity 40% (95% CI 7-83)).

Table 5.12 Cross-tabulations of the modified Boston criteria classifications using probable CAA or possible/probable CAA as the index test cut off against the histopathological reference standard

Modified Boston criteria (Index test)	**Vonsattel ≥2 (Reference standard)**		
	No CAA	CAA	Total
No/possible CAA	2	4	6
Probable CAA	3	7	10
Total	5	11	16
No CAA	2	3	5
Possible/probable CAA	3	8	11
Total	5	11	16

CAA = cerebral amyloid angiopathy. DTA = diagnostic test accuracy. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome.

Table 5.13 DTA statistics for the modified Boston criteria CAA using probable CAA or possible/probable CAA as the index test cut off

	Modified Boston criteria	
	Probable CAA	Possible/probable CAA
Sensitivity	64 (32-88)	73 (39-93)
Specificity	40 (7-83)	40 (7-83)
Positive likelihood ratio	1.1 (0.5-2.5)	1.2 (0.5-2.7)
Negative likelihood ratio	0.9 (0.2-3.6)	0.7 (0.1-3.2)
Positive predictive value	70 (35-92)	73 (39-93)
Negative predictive value	33 (6-76)	40 (7-61)

Data are percentage or ratio (95% CI). CAA = cerebral amyloid angiopathy. DTA = diagnostic test accuracy. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome.

5.4.3 Index test versus reference standard in lobar ICH

In a sensitivity analysis I assessed the diagnostic accuracy of the original and modified Boston criteria in those with a lobar ICH. The sensitivity of both the original Boston criteria (Table 5.14 and Table 5.15) and the modified Boston criteria (Table 5.16 and Table 5.17) was higher, however there were no true negative cases in this small group of participants, resulting in a specificity of 0%.

Table 5.14 Cross-tabulations of the original Boston criteria classifications using probable CAA or possible/probable CAA as the index test cut off against the histopathological reference standard in lobar ICH participants

Original Boston criteria (Index test)	**Vonsattel ≥2 (Reference standard)**		
	No CAA	CAA	Total
No/possible CAA	0	3	3
Probable CAA	2	6	8
Total	2	9	11
No CAA	0	1	1
Possible/probable CAA	2	8	10
Total	2	9	11

CAA = cerebral amyloid angiopathy. DTA = diagnostic test accuracy. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome.

Table 5.15 DTA statistics for the original Boston criteria CAA using probable CAA or possible/probable CAA as the index test cut off in lobar ICH participants

	Original Boston criteria			
	Probable CAA		Possible/probable CAA	
Sensitivity	67	(31-91)	89	(51-99)
Specificity	0	(0-80)	0	(0-80)
Positive likelihood ratio	0.7	(0.4-1.6)	0.9	(0.7-1.1)
Negative likelihood ratio	Inf	(NaN-inf)	Inf	(NaN-Inf)
Positive predictive value	75	(36-96)	80	(44-96)
Negative predictive value	0	(0-69)	0	(0-95)

Data are percentage or ratio (95% CI). CAA = cerebral amyloid angiopathy. DTA = diagnostic test accuracy. Inf = infinity. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. NaN = Not a number.

Table 5.16 Cross-tabulations of the modified Boston criteria classifications using probable CAA or possible/probable CAA as the index test cut off against the histopathological reference standard in lobar ICH participants

Modified Boston criteria (Index test)	**Vonsattel ≥2 (Reference standard)**		
	No CAA	CAA	Total
No/possible CAA	0	2	2
Probable CAA	2	7	9
Total	2	9	11
No CAA	0	1	1
Possible/probable CAA	2	8	10
Total	2	9	11

CAA = cerebral amyloid angiopathy. DTA = diagnostic test accuracy. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome.

Table 5.17 DTA statistics for the modified Boston criteria CAA using probable CAA or possible/probable CAA as the index test cut off in lobar ICH participants

	Modified Boston criteria			
	Probable CAA		Possible/probable CAA	
Sensitivity	78	(40-96)	89	(51-99)
Specificity	0	(0-80)	0	(0-80)
Positive likelihood ratio	0.8	(0.5-1.1)	0.9	(0.7-1.1)
Negative likelihood ratio	Inf	(NaN-inf)	Inf	(NaN-Inf)
Positive predictive value	78	(40-96)	80	(44-96)
Negative predictive value	0	(0-80)	0	(0-95)

Data are percentage or ratio (95% CI). CAA = cerebral amyloid angiopathy. DTA = diagnostic test accuracy. Inf = infinity. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. NaN = Not a number.

5.5 Discussion

5.5.1 Main findings

- The probable CAA category in the original Boston criteria showed 55% sensitivity and 60% specificity for CAA-associated ICH (Vonsattel grade ≥2 on research brain autopsy).
- The combination of either possible or probable CAA categories in the original Boston criteria increased sensitivity to 73% for CAA-associated ICH but lowered the specificity to 40%.
- The inclusion of any cortical superficial siderosis as a distinct haemorrhagic lesion (modified Boston criteria) increased the sensitivity of probable CAA to 64% for CAA-associated ICH but decreased the specificity to 40%.
- The combination of either possible or probable CAA categories in the modified Boston criteria increased sensitivity to 73% for CAA-associated ICH without changing the specificity (40%).

5.5.2 Strengths of the study

I performed and reported the study accorded to the STARD guidelines for diagnostic accuracy studies.[173] Important strengths are:

- We used prospective case ascertainment to reduce selection bias by inviting all potentially eligible patients to the study.[175, 287]
- The index test and reference standard were acquired prospectively, and we optimised procedures for the study question.[175]
- We performed the index test at a standardised time point after the index ICH for all participants.
- The reference standard was offered to all eligible participants, regardless of clinical features or the results of the index test to reduce partial verification bias.[174, 176, 246]
- All included participants underwent the same index test and reference standard to avoid differential verification bias.[174]
- I minimised information bias by using a standard MRI acquisition protocol for all participants and I followed published guidance for rating

SVD biomarkers on MRI.[77] We also used a standardised approach for systematically acquiring the reference standard[217] and a validated scale to evaluate CAA.[36] Assessors of the index test and reference standard were masked to relevant features.[175, 176]

- There were no missing data.
- I pre-specified the sensitivity analysis using a different cut-off for index test positivity.[288]
- I reported the flow of participants through the study and described the differences between those who did and did not undergo the reference standard to illustrate partial verification bias.[174, 175]
- I described the baseline clinical and radiographic features and the distribution of CAA and non-CAA SVDs severity in the study groups to describe the spectrum of participants.[175, 176]

5.5.3 Weaknesses of the study

Although we tried to limit selection bias, those included in the study were older than those not included, and they tended to have a smaller ICH volume and a less severe CT SVD score. These variations in the spectrum of disease can bias the diagnostic accuracy of a test,[176] although the differences were small. The older age of included participants may relate to the increased likelihood of dying after ICH with increasing age[244] and thus undergoing the autopsy reference standard. The other differences probably reflect the feasibility of MRI scanning in this patient group.

The sample size was small, which was related to the rigorous reference standard I used. Only 16 of the 160 participants who underwent the index test had the reference standard. Patients able to undergo MRI tend to be less severely affected by ICH than those not able to have MRI (Table 3.10). As a result, the fatality rate will be lower in participants with the index test versus those without. Acquisition of an autopsy reference standard in those with the index test was therefore infrequent. The small sample size results in less precise estimates of diagnostic accuracy and reduces generalisability.[289] The lack of power also prevented me from assessing independent

associations between clinical, genetic and MRI SVD biomarkers and pathologically-proven CAA. However, a further 79 participants who have undergone research MRI have consented to research brain autopsy, 67 of whom are still alive, so more data will become available with the passage of time.

The use of an autopsy-based reference standard resulted in two further limitations. Firstly, most participants who had the index test did not undergo the reference standard (Figure 5.1 and Figure 5.2), resulting in a potential partial verification bias. Partial verification bias is common in studies using an invasive reference standard.[290] All LINCHPIN participants were invited to provide consent to research autopsy, and those who consented to this were similar to those who did not. However, those who underwent autopsy in this study were older with more frequent pre-ICH dementia compared to those only having the index test (Table 5.2). Although the index test result did not influence the reason for undergoing an autopsy, the prevalence and severity of CAA in my study is likely to be increased relative to the population of interest given the association between CAA and increasing age and dementia.[21, 27, 233, 258] In line with this, the ratio of positive to negative index test results according to both the original and modified Boston criteria was higher in those undergoing an autopsy. This is likely to bias the studies by increasing the sensitivity of the criteria.[174, 176, 291]

Secondly, the use of autopsy leads to a time delay between the index test and reference standard. Ideally, the index test and reference standard should be performed at the same time or as close as possible.[173, 292] The median time between the MRI and autopsy was 927 days and ranged from 44 days to 2405 days. CAA is a progressive, age-related condition.[27, 258] Therefore, a delay in performing the histopathological assessment may lead to a false positive reference standard classification due to the interval development of CAA after the index test was performed. This would reduce index test sensitivity. Four of the false negative index test results for the original Boston criteria when using probable CAA as the index test cut-off

occurred in elderly participants whose autopsy occurred over a year after the index test (Figure 5.8). Three of these participants were also false negative results in the modified Boston criteria diagnostic test accuracy study.

5.5.4 Comparison with other studies

The sensitivity of the original Boston criteria was 55% (95% CI 25-82) when I used the probable CAA category as the cut-off. This is lower than the original study characterising the Boston criteria by Knudsen et al. (73% (95% CI 39-93))[103] and external validation studies by Charidimou et al. (77% (95% CI 46-95))[95] and Linn et al. (90% (95% CI 76-96)).[110] However, the studies by Knudsen et al[103] and Charidimou et al[95] have confidence intervals which overlap the point estimate from my study.

The probable CAA category is the highest level of diagnostic certainty and therefore should maximise specificity over sensitivity. When I performed a sensitivity analysis using possible or probable CAA categories as the cut-off, the sensitivity of both the original and modified criteria increased to 73% (95% CI 40-93). A similar result was found by Charidimou et al. (sensitivity 92% (95% CI 62-100)).[95] As expected the specificity reduced with this cut-off in both my study (40% (95% CI 7-83)) and the study by Charidimou et al. (63% (95% CI 25-92)).[95]

In my study the specificity of the probable CAA category in the original Boston criteria was 60% (95% CI 17-93). This is lower than the original study characterising the Boston criteria (100% (95% CI 40-100))[103] and the external validation studies (88% (95% CI 47-100)[95] and 81% (95% CI 62-93),[110] but the confidence intervals in these studies overlap with the point estimate from my study.

The inclusion of cortical superficial siderosis as a distinct haemorrhagic lesion in the criteria (modified Boston criteria) increased the sensitivity of the probable CAA category from 55% to 64% (95% CI 32-88). However, the specificity reduced from 60% to 40% (95% CI 7-83). The only previous study assessing the modified Boston criteria also reported an increase in the sensitivity compared with the original Boston criteria (95% (95% CI 83-99)

versus 90% (95% CI 76-96) respectively), but the specificity remained unchanged at 81% (95% CI 62-93).[110]

I included participants with both lobar and non-lobar ICH locations in my primary analysis as this was the approached used by Charidimou et al in their external validation of the original Boston criteria[95] and Linn et al in the development of the modified Boston criteria.[110] I performed a sensitivity analysis excluding participants with non-lobar ICH because there is thought to be no association between CAA and deep ICH[153] and the original Boston criteria were developed in patients with only lobar ICH.[103] As expected there were fewer false-negative results with both the original and modified Boston criteria when applied to only lobar ICH, resulting in a higher sensitivity. However there were only two non-CAA-associated lobar ICH cases, both of which were false positive results using the original and modified Boston criteria.

There are several potential reasons relating to the study methodology and the design of the criteria to explain the differences in diagnostic accuracy between my study and the other studies. The weaknesses in my study and the likely effects on sensitivity and specificity are discussed in section 5.5.3, while those relating to the previous studies are described below.

5.5.4.1 Limitations of previous studies

All three previous studies were small (Knudsen et al. n = 15, Charidimou et al. n= 21, Linn et al. n=60) with wide 95% CI.[95, 103, 110] A pooled analysis of the data could improve the power and refine the estimates of diagnostic accuracy. However, as discussed below, there are major methodological limitations in all these studies, which would make it difficult to interpret any meta-analysis.

The previous studies all identified potential participants through retrospective searches of pathology databases (i.e. the reference standard). This approach can affect the prevalence and severity of the target disease and alternative conditions compared with the population of interest, which can influence the apparent diagnostic accuracy.[287, 293] Indeed, the prevalence of CAA

cases in these studies ranged from 62-74% [95, 103, 110] compared with 61% prevalence of CAA-associated lobar ICH in our LINCHPIN brain bank (Figure 4.13). The prevalence of CAA-associated ICH in my study was also high (69%).

The reference standard was not obtained in all who underwent the index test in the previous studies. Instead, it was performed as part of the clinical management and may have been influenced by the likelihood and severity of CAA, based on the clinical features and the index test result. This leads to partial verification basis, where there is the preferential performance of the reference standard in those with a higher probability of the disease of interest and will result in biased estimates of diagnostic accuracy by falsely increasing sensitivity and decreasing specificity.[174, 176, 291]

All the previous studies used more than one type of reference standard. The accuracy of detecting CAA will differ between brain biopsy, haematoma evacuation and autopsy due to the small volume of tissue sampled by the former two. Analysing the different reference standards together without accounting for this differential verification will result in biased accuracy estimates.[174, 294]

The control groups in all three previous studies were younger than the cases. Given the increasing prevalence of CMBs with age,[99, 295, 296] the chance of false-positive results in such participants will be lower, which may have falsely increased specificity.[175]

The previous studies by Charidimou et al[95] and Linn et al[110] included participants with non-lobar ICH as well as participants without ICH who had undergone the index test and reference standard. This heterogeneous case mix can affect diagnostic accuracy estimates. Also, the previous studies included participants with both first-ever and recurrent ICH. This could falsely increase sensitivity given that participants with a recurrent ICH are likely to have more severe underlying SVDs.[176, 297]

Finally, the studies performed by Charidimou et al[95] and Linn et al[110] did not report whether assessment of the reference standard was masked to clinical information or the index test, which can result in the reported diagnostic accuracy of the index test being too high.[294] The timing between the index test and reference standard can affect diagnostic accuracy, but this was not reported in any of the previous studies.

5.5.4.2 Limitations of the Boston criteria

The Boston criteria may have misclassified participants, affecting its sensitivity. One of the false negative results in my study of the original Boston criteria occurred in a participant classified as possible CAA. They had a single lobar ICH and no microbleeds. However, cortical superficial siderosis, which is known to be associated with CAA,[110] was also present. This negative case was correctly classified when I applied the modified Boston criteria, leading to an increase in the sensitivity.

A difficulty with the original and modified Boston criteria relates to mixed location haemorrhagic foci. The SVDs underlying ICH are typically heterogeneous, with mixed CAA and non-CAA SVDs often present in lobar ICH (Figure 4.13). Any non-lobar haemorrhage results in a “no CAA” classification by both the original and modified Boston criteria, which is potentially problematic in cases where there are multiple lobar haemorrhages, suggestive of CAA, and a single deep CMB. One false negative result in my study occurred in a participant with lobar ICH and multiple lobar CMBs but a single deep CMB. This participant was classified as no CAA by both the original and modified Boston criteria. The autopsy showed severe CAA as well as severe non-CAA SVD.

The lower specificity in my study may relate to misclassification by Boston criteria. One of the false positive results in my study occurred in a participant with a single lobar haemorrhage and two cortical cerebellar CMBs. On this basis, they were classified as probable CAA. However the autopsy showed mild meningeal and absent parenchymal CAA, but severe non-CAA SVD. Cerebellar haemorrhage can occur in both CAA and arteriosclerosis.[153,

298] Therefore the validity of their inclusion as a separate lobar haemorrhagic focus in the Boston criteria is unclear, particularly when the severity of CAA in the cerebellum is often milder compared with the cerebrum (Figure 4.16).[167]

5.5.5 Clinical implications

The original and modified Boston criteria might be useful non-invasive approaches to rule in or rule out CAA-associated ICH. This would limit the need for invasive investigations to diagnose CAA, such as brain biopsy, and could help guide clinical management and inform prognosis after ICH.[59, 105, 158] However, given the methodological limitations of the external validation studies of the original criteria and the lack of external validation of the modified criteria, their true diagnostic accuracy is uncertain. My preliminary data in a small cohort suggest that the diagnostic accuracy of the original and modified Boston criteria is lower than the published values. Therefore, these criteria should be used with caution in clinical practice.

5.5.6 Future directions

Future work should focus on rigorous external validation of the original and modified Boston criteria with a larger sample to quantify their diagnostic accuracy. The ideal external validation study would involve an unselected, population-based cohort study of first-ever lobar ICH comparing standardised MRI imaging with blood-sensitive sequences against a standardised histopathological reference standard acquired close to the index test. The index test result should not influence the decision to perform tissue sampling in order to limit partial verification bias. Imaging and histopathological assessments should be masked.[173]

The Boston criteria have limitations when there are mixed location haemorrhages or a solitary lobar haemorrhage. Therefore, future studies should aim to improve the Boston criteria. This could be through the development of a multivariable prediction model for CAA. Such a model should include relevant clinical features, such as age and pre-existing dementia, APOE genotype and MRI biomarkers, such as the number of lobar

and non-lobar macrohaemorrhages and CMBs, the extent of cortical superficial siderosis and other more recently identified CAA-associated MRI biomarkers. This approach allows the probability of CAA to be predicted based on the presence and number of these predictors, helping to circumvent the problems described above. Simple criteria could be derived from this model, and relevant clinical cut-offs identified to rule in or out CAA.

The LINCHPIN study contains many of the ideal characteristics of a diagnostic test accuracy study. Sixty-seven study participants who had a research MRI and consented to research autopsy are still alive. Repeating my analyses when these participants have died and donated brain tissue at research autopsy will provide a more robust assessment of the performance of the Boston criteria and an opportunity to develop a novel MRI-based prediction model for CAA-associated ICH. However, the sample size will still be modest.

The International CAA Association is currently coordinating a multicentre study to validate and update the Boston criteria.[228] This study will include participants from different centres around the world and will be the largest study of its kind in CAA. It will have limitations, such as heterogeneous index tests (MRI field strength, scanner type and sequence parameters), heterogeneous reference standards (haematoma evacuation, brain biopsy and autopsy) and possible selection bias favouring participants with CAA. However, it will present a good opportunity to develop and validate MRI-based diagnostic criteria for CAA.

Chapter 6 Diagnostic value of β-amyloid PET in SVD-associated ICH

6.1 Introduction

The modified Boston criteria are the current non-invasive *in vivo* reference standard for diagnosing CAA.[110, 167] These criteria use the presence of haemorrhagic consequences of CAA on MRI (macrohaemorrhages, CMBs, and cortical superficial siderosis) to categorise a patient as probable CAA, possible CAA or no CAA. However, haemorrhages are thought to represent the late consequences of advanced CAA.[170] Therefore, those in the earlier stages of the disease spectrum, in whom haemorrhages have not yet occurred, may not be identified. In addition, multiple sites of haemorrhage are necessary to diagnose "probable" CAA on the modified Boston criteria, yet 27% of ICH patients had a single lobar index ICH on MRI in a hospital-based study,[243] while 36% of patients in my population-based LATCH study have a single lobar index ICH identified on CT +/- MRI (Figure 3.2). The significance of this possible CAA category in relation to underlying CAA and risk of future haemorrhages is unclear. A more accurate diagnostic approach is therefore essential.

In vivo molecular imaging with PET is a technique that may help identify CAA. The direct detection of perivascular β-amyloid with PET may allow accurate diagnosis of CAA even before haemorrhages have occurred.

^{11}C-Pittsburgh compound B (PiB), a benzothiazole dye that binds β-amyloid, was initially developed as a PET radioligand for fibrillar/parenchymal β-amyloid in Alzheimer's disease. PiB is now recognised to be a non-specific β-amyloid ligand, binding with high affinity to both non-vascular parenchymal and perivascular β-amyloid.[171, 172] Three studies have demonstrated that PiB uptake in patients with possible CAA or probable CAA according to the modified Boston criteria (n=60) is significantly higher than healthy controls without ICH (n=87),[252, 262, 299] while other studies have shown PiB

uptake corresponds topographically to lobar microbleeds.[300, 301] The distribution of PiB uptake in possible CAA or probable CAA also differed from that observed in Alzheimer's disease (n=22).[252, 262] In contrast, one study did not show a difference in PiB uptake between probable CAA-related ICH (n=11) and healthy controls (n=9).[302] These conflicting results perhaps reflect the small sample sizes combined with other methodological limitations, such as heterogeneous case groups including those with and without ICH, poorly matched control groups and variable techniques used for PET data analysis.

The short half-life of ^{11}C-PiB (20 minutes) restricts its use outside the research setting. The newer ^{18}F-labelled amyloid PET ligands, such as ^{18}F-flutemetamol, have a half-life six times longer, making them more practical for clinical practice.

Two recent studies have shown that uptake of florbetapir, an ^{18}F-amyloid tracer, is significantly higher in probable CAA lobar ICH (n=25) versus deep ICH controls (n=27).[303, 304] Flutemetamol is a structural analogue of PiB, and the two compounds possess very similar *in-* and *ex-vivo* binding characteristics in Alzheimer's disease and healthy controls.[305] Therefore, ^{18}F-flutemetamol is expected to be a non-specific β-amyloid ligand. However, whether it binds to perivascular β-amyloid, and whether ^{18}F-flutemetamol can identify CAA *in vivo* is uncertain.

6.2 Aims

I aimed to:

- Assess whether 6-CN-flutemetamol, a fluorescent derivative of flutemetamol, labels perivascular (CAA) and non-vascular parenchymal β-amyloid using *ex vivo* brain tissue samples from participants with SVD-associated ICH.
- Perform a diagnostic test accuracy study of ^{18}F-flutemetamol PET for CAA-associated ICH, comparing the visual classification of ^{18}F-

flutemetamol PET scans (index test) against the MRI-based modified Boston criteria (reference standard).

- Compare visual evaluation of the ^{18}F-flutemetamol PET scans against quantitative analysis of cortical standardised uptake value ratios (SUVr) in SVD-associated ICH.
- Quantitatively assess the amount and distribution of ^{18}F-flutemetamol uptake between probable CAA versus possible CAA or no CAA according to the modified Boston criteria in patients with ICH.

6.3 Methods

6.3.1 *Ex vivo* 6-CN-flutemetamol study

6.3.1.1 Selection of cases

I reviewed the histopathological ratings for CAA[36] and non-vascular (Alzheimer's type) β-amyloid (Thal phase)[219] in the LINCHPIN brain bank to identify four cases covering the extremes of CAA and non-vascular β-amyloid severity.

- Absent parenchymal and meningeal CAA and absent non-vascular β-amyloid (Thal phase 0).
- Absent parenchymal and meningeal CAA and severe non-vascular β-amyloid (Thal phase 5).
- Severe parenchymal and meningeal CAA and absent non-vascular β-amyloid (Thal phase 0).
- Severe parenchymal and meningeal CAA and severe non-vascular β-amyloid (Thal phase 5).

6.3.1.2 Immunofluorescence

The immunofluorescence was performed by Karina McDade (KM, Senior Research Technician, Centre for Clinical Brain Sciences, The University of Edinburgh) and Dr Juraj Koudelka (JK, Research Fellow, Centre for Discovery Brain Sciences, The University of Edinburgh).

KM cut one 8 μm section from a formalin fixed paraffin embedded frontal convexity (BA46) tissue block from each participant using a microtome. She mounted the sections onto glass slides and fixed them by heating at 40°C overnight. The frontal cortex was selected as it is known to be affected by both CAA and non-vascular β-amyloid.

JK heated the sections to 60°C for 60 minutes to melt the wax. He deparaffinised the sections by washing in xylene twice (15 minutes per wash) and hydrated them in two changes of 100% ethanol (five minutes each), 90% ethanol for one minute and 70% ethanol for one minute. JK washed the sections using distilled water and then equilibrated them in phosphate buffered saline (PBS) for five minutes.

JK performed antigen retrieval using citrate buffer heat retrieval. He placed the slides in a rack filled with 10mM citrate buffer brought to pH 6.0 using 2M NaOH then added 0.25 ml Tween 20. The rack was placed in an antigen retrieval machine (Biocare Decloaking Chamber NxGen, California, USA) and heated to 95°C for ten minutes.

Once cooled, JK equilibrated the sections by washing in PBS twice (five minutes per wash) then PBS-Tween 20 (0.05%) twice (two minutes per wash) before blocking them with 0.5% bovine serum albumin (BSA) and 10% normal serum in PBS for one hour at room temperature. He then drained excess block and incubated the sections with primary antibodies for Collagen type IV to label the vascular basement membrane of blood vessels (Col IV; 1:1000, 70R-CR013x; Fitzgerald Industries Intl, MA, USA) and β-amyloid (6E10; 1:1000, SIG-39320; Signet/Covance, Dedham, MA, USA) at 4°C overnight in blocking buffer.

On the next day, JK washed the sections twice in PBS-Tween 20 (0.05%) (ten minutes per wash) before incubating them for two hours at room temperature in the dark with fluorescent secondary antibodies (Col IV: goat anti-rabbit Alexa Fluor 546, Thermofisher; 6E10: goat anti-mouse Alexa Fluor 488, Thermofisher, both diluted 1:500 in PBS) and 6-CN-flutemetamol (courtesy of Dr Milos Ikonomovic, University of Pittsburgh) (1 μM solution

made with 0.1M KPBS). He then washed the sections twice in PBS (ten minutes per wash) and once in Tris Buffer (10 minutes). The sections were air-dried at room temperature for ten minutes. He mounted coverslips using VECTASHIELD Hardset (VECTOR Laboratories, UK) and sealed the edges with nail varnish.

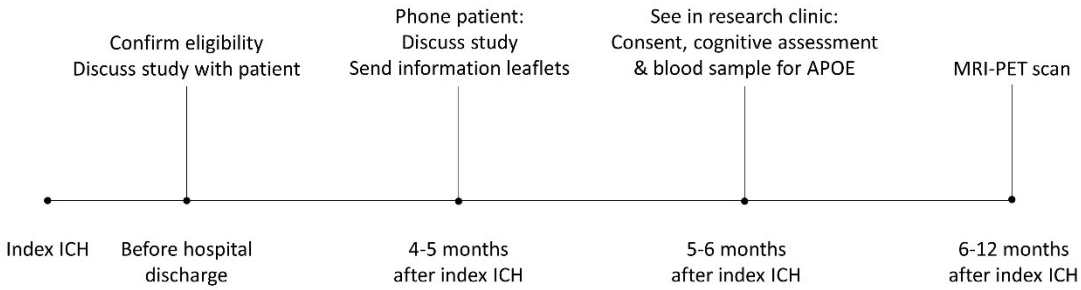
We imaged the sections using a laser scanning confocal microscope Zeiss LSM 710 (Oberkochen, Germany) using 10x and 20x lenses.

6.3.2 *In vivo* ^{18}F-flutemetamol MRI-PET studies

6.3.2.1 Study design and participants

I prospectively identified incident cases of ICH in NHS Lothian between 1st December 2015 and 26th September 2018 (Figure 6.1). I reviewed NHS Lothian's electronic records system twice a week to identify all patients with ICH admitted to NHS Lothian stroke units, as well as those seen in stroke/TIA clinics. I included consecutive adults aged 40-95 years who had a first-ever ICH, were resident in the NHS Lothian Health Board region and provided consent.

Figure 6.1 MRI-PET study timeline



APOE = apolipoprotein E. ICH = intracerebral haemorrhage. MRI = magnetic resonance imagine. PET = positron emission tomography.

I excluded patients with exclusively extra-axial intracranial haemorrhage, ICH secondary to an underlying cause other than SVDs, infratentorial ICH and

recurrent ICH. I also excluded patients if they had a contraindication to MRI, such as a non-MRI compatible implantable device or metallic foreign bodies in the eye, were unable to tolerate MRI-PET scanning because they were claustrophobic, unable to transfer independently onto the MRI scanner, unable to lie flat, too unwell or unable to fit into the scanner, and those with a known diagnosis of dementia.

6.3.2.2 Baseline data collection

I collected demographics, the presence of relevant co-morbidities and medication use at the time of ICH by interviewing participants and their relatives, and reviewing medical records as described in section 2.1.7.

6.3.2.3 Cognitive assessment

I used the mini mental state examination (MMSE), Montreal cognitive assessment (MoCA) and Addenbrooke's cognitive examination (ACEIII) to assess cognition before the MRI-PET scan because cognitive impairment and dementia are associated with β-amyloid deposition so they could be confounders for amyloid PET uptake associated with ICH location.[306, 307]

6.3.2.4 Index test

MRI-PET acquisition

The Edinburgh Imaging Facility QMRI, The University of Edinburgh, performed ^{18}F-flutemetamol brain MRI-PET scans using a hybrid 3T mMR Biograph (Siemens Medical Solutions, Erlangen, Germany) around six to 12 months after the ICH. The Edinburgh Imaging Facility QMRI radiochemistry laboratories produced ^{18}F-flutemetamol under good manufacturing practice conditions.

The scan protocol consisted of simultaneous MRI and PET acquisition following the intravenous injection of 185MBq ^{18}F-flutemetamol. The first 30-minute scan started when a 185MBq bolus of ^{18}F-flutemetamol was injected via an antecubital vein and included 30 minutes of dynamic PET emission scanning in 3D list mode (voxel size 2.3 x 2.3 x 5.0 mm, signal to noise ratio 1). The following MRI sequences were performed simultaneously: MR Attenuation Correction (MRAC), MRAC-Ultrashort TE (UTE), Arterial Spin

Labelling (ASL), 3D T1-weighted, T2-weighted and FLAIR sequences, and SWI.

The second 30-minute window started at 90 minutes after ^{18}F-flutemetamol injection and included 30 minutes of dynamic PET emission scanning in 3D list mode as above plus simultaneous acquisition of the following MRI sequences: MRAC, MRAC-UTE, 3D T1-weighted, axial T2-weighted, FLAIR and T2*-weighted GRE, and DWI/diffusor tensor imaging (DTI) sequences.

The MRI sequence parameters are shown in Table 6.1.

I reconstructed ^{18}F-flutemetamol PET scans with 3D iterative reconstruction with three iterations using the MRAC-UTE for attenuation correction. Corrections for randoms, dead time, normalisation, scatter and sensitivity were automatically applied.

Table 6.1 MRI sequence parameters in the MRI-PET study

	ASL	3D-T1	3D T2	Axial T2	3D FLAIR	Axial FLAIR	SWI	Axial T2* GRE	DTI
Orientation	AC-PC	SAG	SAG	AC-PC	SAG	AC-PC	AC-PC	AC-PC	AC-PC
TE (ms)	11	2.26	294	117	398	133	20	19.9	81
TR (ms)	2500	2400	2800	5500	5000	9000	28	620	8300
TI (ms)		900			1800	2500			
Flip angle (degrees)	90	8		90		130	9	20	
Field of view (mm^2)	192	256	250	220	250	220	240	220	250
Slice thickness (mm)	6	1	1	4	1	4	1.7	4	3
Slice gap (mm)				0		4		4	
Diffusion directions									39
b-value 1 (s/mm^2)									0
b-value 2 (s/mm^2)									750

AC-PC = anterior commissure-posterior commissure. DTI = diffusion-tensor imaging. FLAIR = fluid-attenuated inversion recovery. GRE = gradient echo. MRI = magnetic resonance imaging. SAG = sagittal. SWI = susceptibility weighted imaging. TE = echo time. TI = inversion time. TR = repetition time.

Qualitative flutemetamol PET image analysis (index test)

Dr Gerard Thompson (consultant neuroradiologist) and I (trainee neuroradiologist) independently assessed ^{18}F-flutemetamol uptake on the reconstructed and corrected PET images derived from the 90 to 120 minutes post-injection interval fused to the participant's 3D T1-weighted images using Syngo Via software (Siemens Medical Solutions, Erlangen, Germany). We performed the assessments masked to clinical, CT and MRI features.

We followed the guidance provided by GE Healthcare for ^{18}F-flutemetamol visual assessment for image alignment, colour scales, and windowing, and completed the Vizamyl™ (flutemetamol F18) Electronic Training Programme before assessing the PET scans.[308]

We re-aligned scans into standard planes (Figure 6.2)

- Axial: Perpendicular to the frontal and occipital poles at the level of the anterior and posterior corpus callosum in the sagittal plane, and parallel to the inferior aspect of the temporal poles in the coronal plane.
- Coronal: Parallel to the frontal and occipital poles in the sagittal plane and perpendicular to the interhemispheric fissure in the axial plane.
- Sagittal: Parallel to the interhemispheric fissure in both coronal and axial planes.

We used a rainbow-PET colour scale, with the pons as the high-intensity reference region set to 90% of maximum (Figure 6.3). We assessed the five standard regions (frontal lobe and anterior cingulate, posterior cingulate and precuneus, lateral temporal lobe, parietal lobe, and striatum) for flutemetamol uptake (Figure 6.4).[308] Also, we assessed uptake in the occipital lobe given the presumed posterior predominance of CAA.[27, 252, 262] We rated the left and right hemispheres separately.

As shown in Figure 6.4, we rated the frontal lobe and anterior cingulate, lateral temporal, parietal and occipital lobes as positive if there was increased cortical uptake, resulting in a convex appearance with loss of the sulcal pattern and/or a steep gradient of intensities from the cortex to the

subarachnoid CSF. We rated the posterior cingulate and precuneus as positive if there was increased cortical uptake. We assessed the striatum as positive if there was contiguous increased uptake between the thalamus and frontal white matter (loss of the “striatal gap”) (Figure 6.4). We classified each region as either positive, negative or equivocal. We classified scans as positive overall if there was at least one positive region. Otherwise, we classified them as negative. We reviewed any cases where we disagreed and came to a consensus decision. I chose a visual assessment of the flutemetamol scans to define index test outcome as this reflects clinical practice. I used the consensus decision for overall positive/negative to determine index test positivity/negativity.

Figure 6.2 Realignment of fused ^{18}F-flutemetamol-3D T1-weighted images into standard planes

A-C. Axial, coronal and sagittal planes before realignment.

D-F. Realigned axial, coronal and sagittal planes. The pink lines show the alignment of the axial plane, the purple lines the alignment of the coronal plane and the orange lines show the alignment of the sagittal plane.

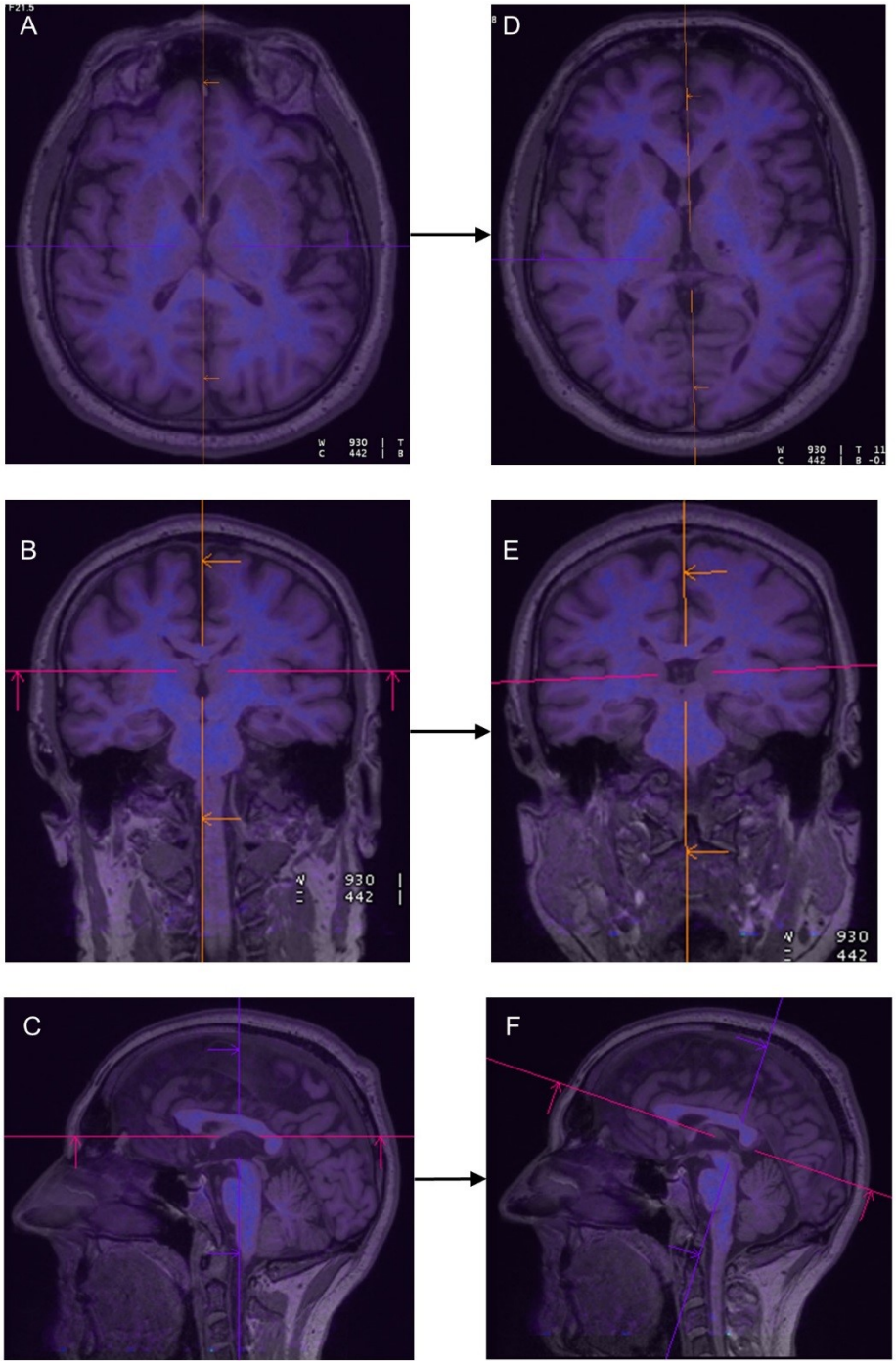
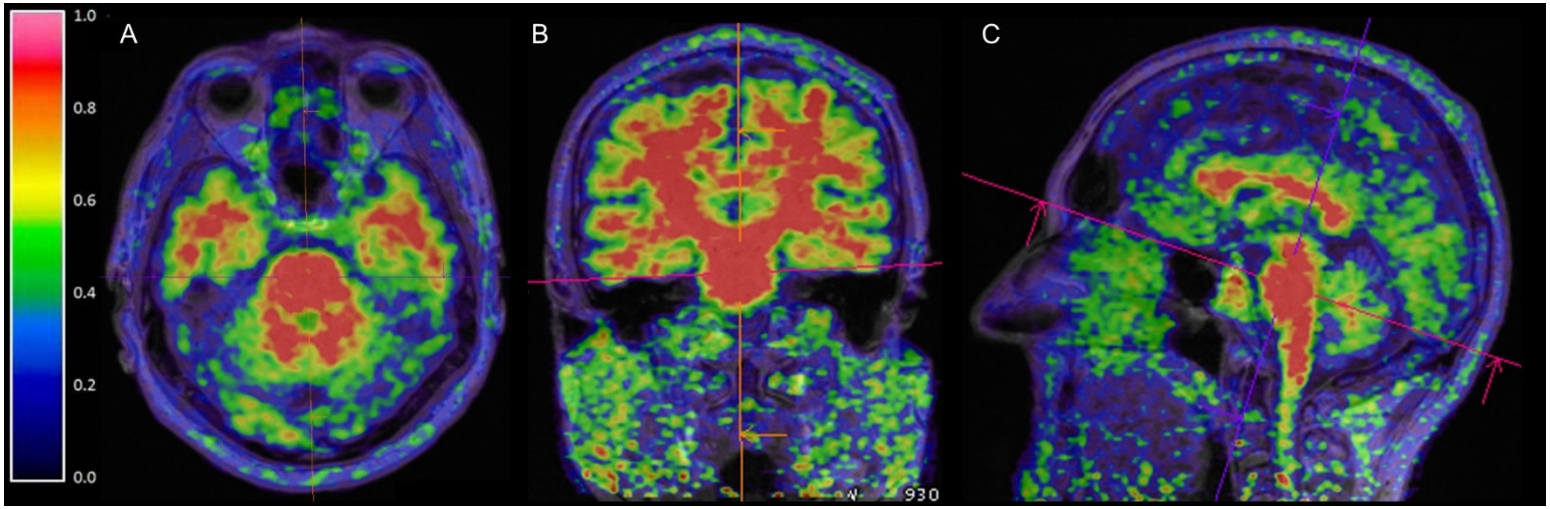


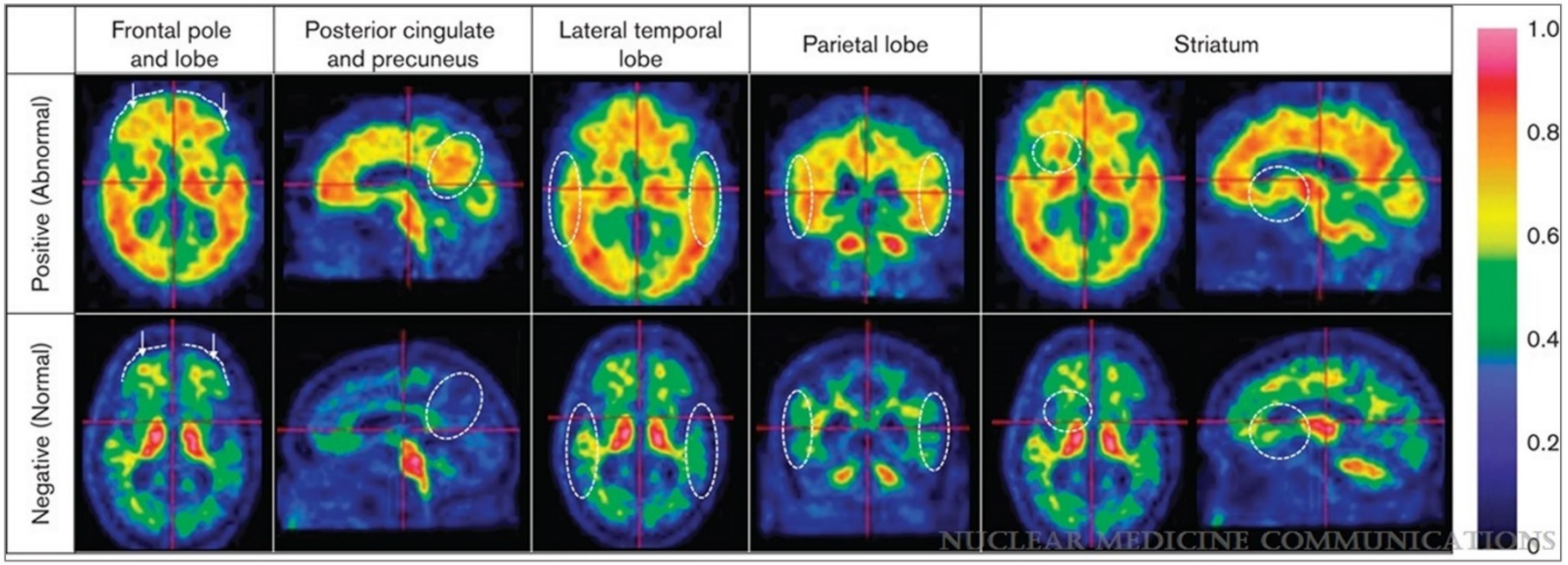
Figure 6.3 Scaling of the rainbow-PET colour scale - the pons is set to 90% of maximum.



PET = positron emission tomography.

Figure 6.4 The five standard regions for flutemetamol uptake showing the features in each region required to make a positive scan classification.

Frontal pole and lobe: the lack of a marked sulcal pattern (dotted lines) and/or sharp intensity gradient from grey matter to cerebrospinal fluid. Posterior cingulate and precuneus: presence of cortical uptake in the circled region. Lateral temporal lobe: heightened uptake throughout and loss of the gyral/sulcal pattern (dotted circles). Parietal lobe: high uptake and decreased sulcal pattern within the dotted circles. Striatum: >50% uptake in the dotted region between the thalamus and the frontal lobe (axial or sagittal view). Reproduced with permission from [309]



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Quantitative flutemetamol PET image analysis

I performed quantitative analysis of the 90 to 120-minute post-injection reconstructed and corrected PET images using PMOD version 3.8 (PMOD Technologies LCC, Zurich, Switzerland).

I spatially normalised the 3D T1-weighted images acquired in the 90-120 minute scanning window to a T1-weighted Montreal Neurological Institute (MNI) brain template. I used the same transformation to normalise the PET images to the T1-weighted MNI brain template. I segmented the normalised T1 images to produce grey matter, white matter and cerebrospinal fluid probability maps.

I defined standard volumes of interest (VOIs) on the normalised PET images using automated anatomical labelling VOIs: frontal lobe, including the insula and anterior and mid cingulate gyrus, parietal lobe including the posterior cingulate gyrus, temporal lobe, occipital lobe and cerebellar cortex. I used the grey matter probability map with a threshold of 75% to restrict the cortical and cerebellar VOIs to grey matter. I created a global cortical VOI by summing all cerebral cortical VOIs. To avoid bias, I did not include any VOIs affected by ICH in this global cortical VOI. I calculated the occipital/global cortex, frontal/global cortex and occipital/frontal ratios to assess for an anterior-posterior CAA gradient, as described in a previous study.[302]

In my primary analysis I used the cerebellar cortex as the reference region to derive regional and global cortical SUVr. Because CAA can affect the cerebellum (Figure 4.16 and Figure 4.17), I compared the cerebellar cortical SUV between probable CAA and possible CAA or no CAA groups. I also manually defined a spherical VOI with a radius of 8mm in the centre of the pons on each scan, ensuring the VOI was contained entirely within the pons, and repeated the analyses using this as the reference region.

6.3.2.5 Reference standard

The ideal reference standard for CAA is a histopathological assessment. However, this is rarely performed during life. Instead, I used the modified

Boston criteria as the reference standard because they are the current non-invasive *in vivo* reference standard for diagnosing CAA.[167]

I rated the MRI brain scans for the presence of acute ICH and ischaemia, chronic ischaemic cortical infarcts, lacunes, chronic haemorrhage and SVD biomarkers using the standardised pro forma described in the methods chapter (Appendix 2). I used the SWI images to categorise scans as probable, possible or no CAA according to the modified Boston criteria.[110] I pre-specified the positive reference standard cut off as probable CAA (CAA-associated ICH) and the negative reference standard cut-off as no CAA or possible CAA (non-CAA-associated ICH) as this is the key diagnostic cut-off used in clinical practice and in research.[110, 167]

I performed all assessments masked to clinical and CT features. I performed the MRI ratings at least three months after the corresponding flutemetamol PET ratings to reduce bias. I performed MRI ratings using Carestream Vue PACS version 11.3.2, Carestream Health, Inc, USA.

6.3.2.6 Statistical analysis

I compared the frequency of clinical features in participants classified as CAA-associated ICH versus not CAA-associated according to the modified Boston criteria reference standard using χ^2 test (or Fisher's exact test, where appropriate) for categorical variables and the Mann-Whitney U test for non-normally distributed continuous variables.

I assessed inter-rater agreement of PET visual ratings using unweighted Cohen's κ coefficient. I evaluated the diagnostic accuracy of visual flutemetamol assessment using sensitivity, specificity, likelihood ratios, and predictive values and their 95% CI. I compared the regional and global SUVr between CAA-associated ICH and not CAA-associated ICH groups using the Mann-Whitney U test as the data were non-normally distributed.

I performed statistical analyses using R statistical package version 3.4.4., except for the diagnostic accuracy statistics, for which I used VassarStats Clinical Calculator 1.[269]

6.3.2.7 Missing data

No data were missing.

6.3.2.8 Sample size

No studies have assessed the difference in ^{18}F-flutemetamol binding between lobar and deep ICH. Previous studies comparing PiB uptake in patients with possible/probable CAA (as defined by the Boston criteria) and controls demonstrated statistically significant differences in mean global distribution volume ratio (DVR) of 0.14 to 0.16 between patients with possible/probable CAA and controls.[252, 299] The standard deviation of DVR for cases ranged from 0.06 to 0.2 and 0.1 to 0.29 for controls.

Using a two-sided, two-sample test with a 5% level of significance, a total sample size of 45 and a ratio of 2:1 case: control [i.e. 30 lobar ICH:15 deep ICH], the minimum detectable difference in means will be 0.14 at 80% power and 0.16 at 90% power. Based on previous studies I expected the common standard deviation to be approximately 0.15. Therefore, I expected to have a sufficient sample size with 45 participants to identify any real differences between the groups.

6.3.2.9 Regulatory approval

The MRI-PET study was approved by the Scotland A Research Ethics Committee (16/SS/0111). I obtained written informed consent from all participants.

6.4 Results

6.4.1 *Ex vivo* 6-CN-flutemetamol study

The clinical and histopathological characteristics of the four LINCHPIN brain bank participants are described in Table 6.2.

6-CN-flutemetamol labels both non-vascular β-amyloid plaques (Figure 6.5 - no CAA and Thal phase 5 case and severe CAA and Thal phase 5 case) and perivascular β-amyloid (Figure 6.5 – severe CAA and Thal phase 0 case and severe CAA and Thal phase 5 case). Both parenchymal and meningeal CAA are labelled by 6-CN-flutemetamol (Figure 6.5 - severe CAA and Thal phase 0 case). There is little non-specific labelling with 6-CN-flutemetamol (Figure 6.5 – no CAA and Thal phase 0 case).

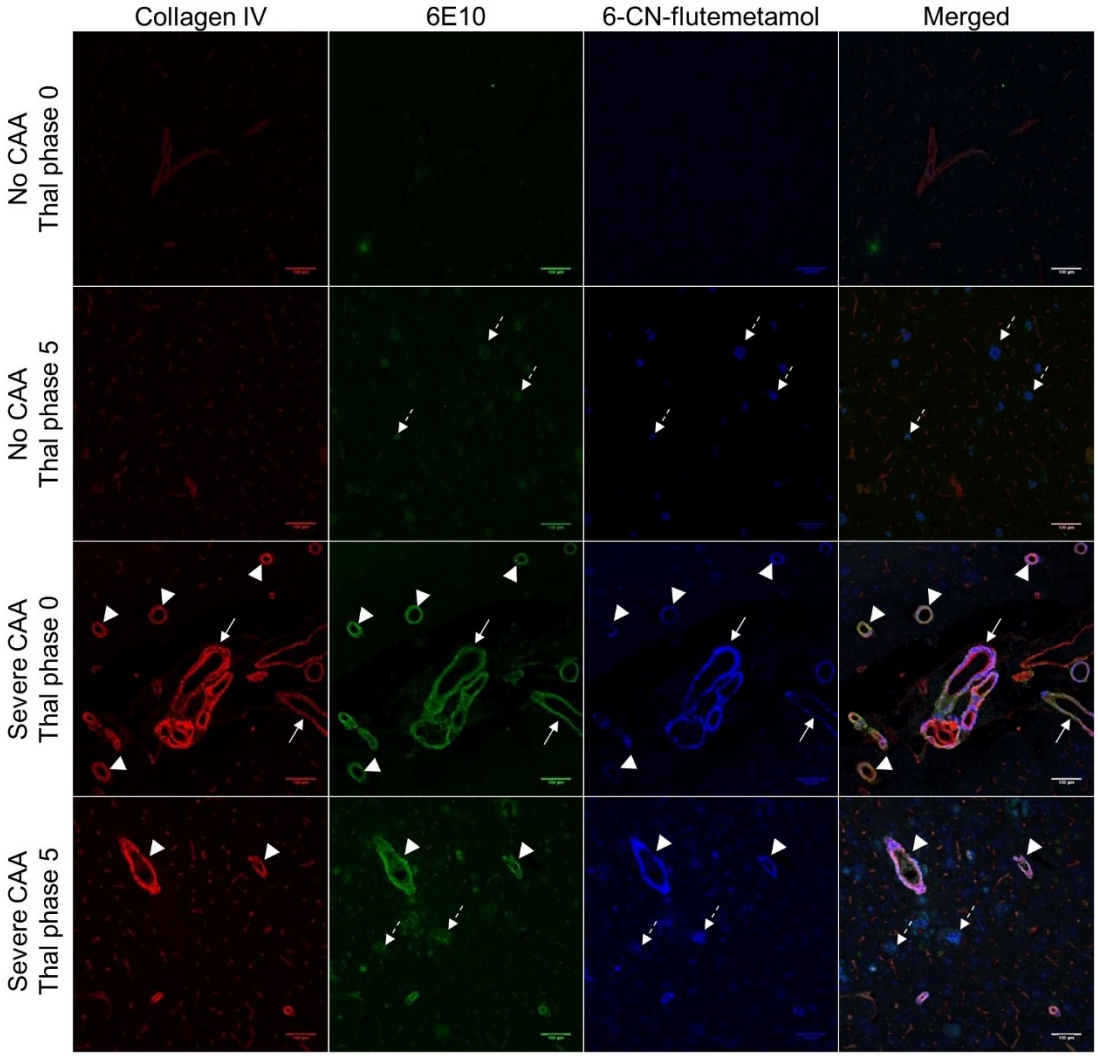
Table 6.2 Clinical and histopathological features of LINCHPIN brain bank participants used in the ex vivo 6-CN-flutemetamol study

	Absent CAA, Thal phase 0	**Absent CAA, Thal phase 5**	**Severe CAA, Thal phase 0**	**Severe CAA, Thal phase 5**
Age at index ICH (years)	56	53	79	81
Sex	Female	Male	Female	Female
Co-morbidities				
Hypertension	No	No	Yes	No
Ischaemic stroke	No	No	No	No
Transient ischaemic attack	No	No	No	No
Dementia	No	No	No	No
Diabetes	No	No	No	No
Atrial fibrillation	Yes	No	No	No
Myocardial infarction	No	No	No	No
Hyperlipidaemia	No	No	No	No
Medications on admission				
Antiplatelet drug(s)	No	No	Yes	No
Anticoagulant drug(s)	No	No	No	No
Antihypertensive drug(s)	Yes	No	Yes	No
APOE genotype	ε3/ε3	ε3/ε3	ε2/ε2	ε3/ε4
ICH details				
Previous ICH	No	No	No	No
Multiple acute ICH	Yes	Yes	No	Yes
Acute ICH location(s)	Occipital lobe Temporal lobe	Basal ganglia Basal ganglia	Frontal lobe	Frontal lobe Parietal lobe
Histopathology features				
Parenchymal CAA severity	Absent	Absent	Severe	Severe
Meningeal CAA severity	Absent	Absent	Severe	Severe
Non-CAA SVD severity	Moderate	Mild	Mild	Moderate
Thal phase	0	5	0	5
Braak Stage	0	6	0	6

APOE = Apolipoprotein. CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and eurological outcome. SVD = small vessel disease.

Figure 6.5 Representative immunofluorescence images for Collagen IV (blood vessels), 6E10 (β-amyloid) and 6-CN-flutemetamol in the frontal convexity in four participants from the LINCHPIN brain bank.

CN-flutemetamol labels perivascular (parenchymal (white arrowheads) and leptomeningeal (white arrows) CAA) and non-vascular β-amyloid (white dashed arrows).



Scale bar = 100 μm. CAA = cerebral amyloid angiopathy. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome.

6.4.2 *In vivo* ^{18}F-flutemetamol MRI-PET studies

6.4.2.1 Participants

Flow of participants

I identified 198 patients with spontaneous ICH between 1st December 2015 and 26th September 2018. Seventy-nine were eligible, of whom I included 20 in the study (Figure 6.6). This is below the target sample size of 45. The predicted versus actual study recruitment is shown in Figure 6.7.

The median time between ICH and MRI-PET (index test and reference standard) was 305 days (IQR 240-350 days, range 190-403 days).

Baseline clinical characteristics of included participants

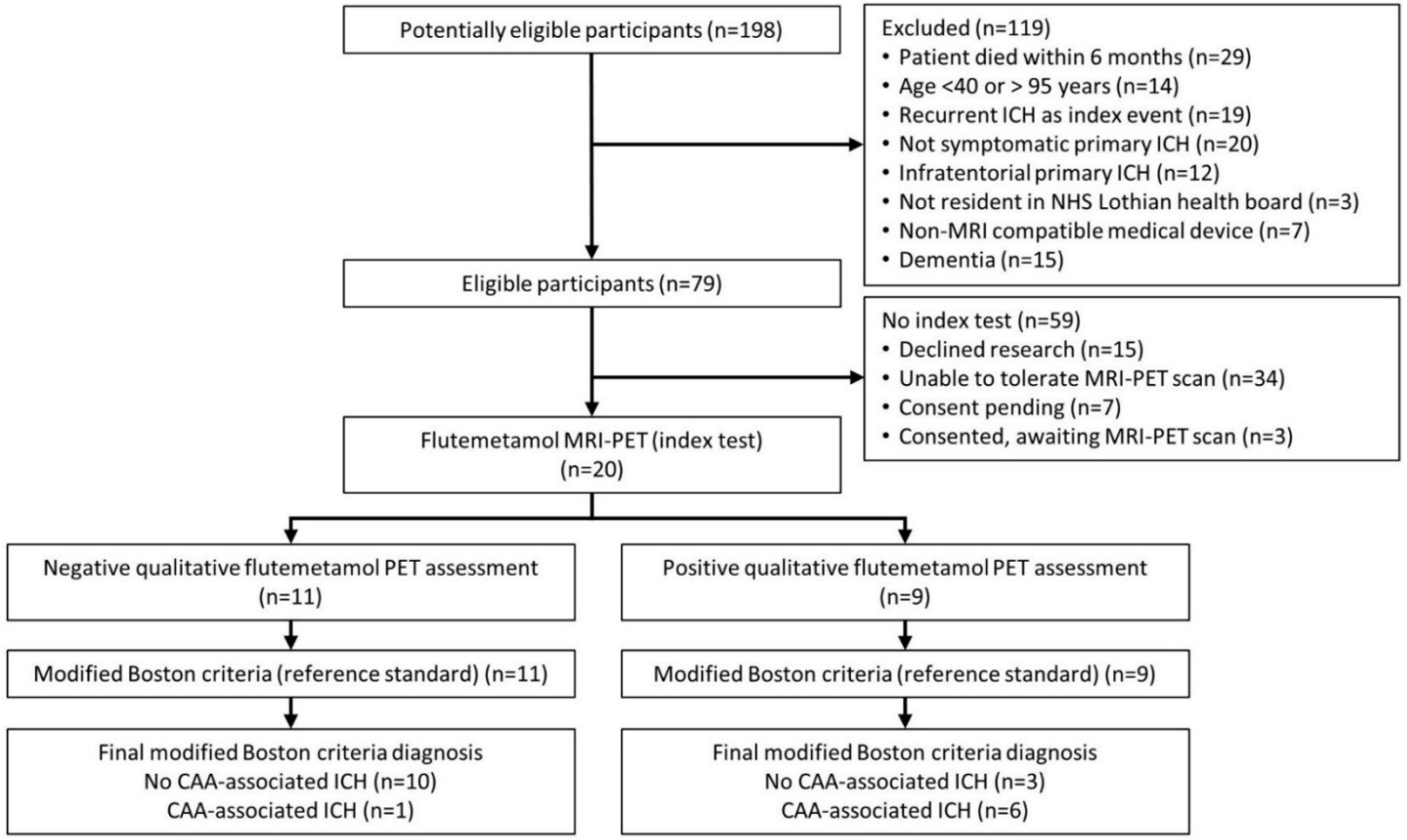
The median age of participants was 71 years (IQR 62-74), and six (30%) were female. All participants were independent before the index ICH (modified Rankin scale 0 or 1) and presented with small symptomatic ICH (median ICH volume 4 cm^3, IQR 2-17). Median admission GCS was 15 (IQR 14-15) (Table 6.3).

Baseline clinical characteristics associated with probable CAA (CAA-associated ICH) versus no CAA or possible CAA (non-CAA-associated ICH)

Seven participants were classified as probable CAA on the modified Boston criteria ("CAA-associated ICH"), 12 as no CAA plus one as possible CAA (grouped as "non-CAA-associated ICH") (Table 6.4).

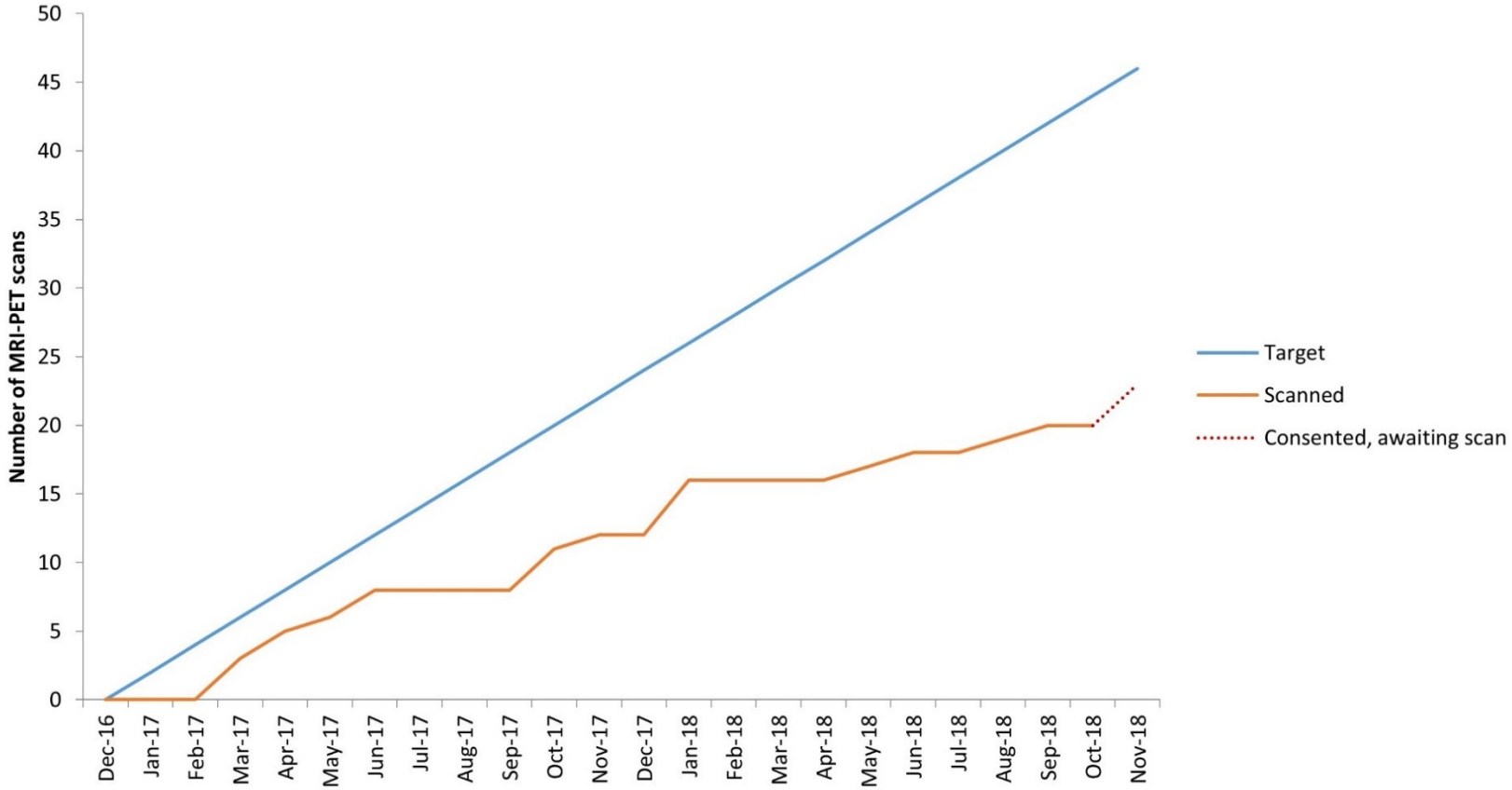
The CAA-associated ICH group were slightly older (median age 73 versus 69) and with a higher proportion of females (43% versus 23%) compared with non-CAA-associated ICH, although these differences did not reach statistical significance. The frequency of co-morbidities and antithrombotic drug use on admission, and post-ICH cognition were similar between the groups (Table 6.5).

Figure 6.6 Flow of participants through the ^{18}F-flutemetamol PET versus the modified Boston criteria diagnostic test accuracy study



CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage. MRI = magnetic resonance imaging. PET = positron emission tomography.

Figure 6.7 Target versus achieved recruitment to the ^{18}F-flutemetamol MRI-PET study.



MRI = magnetic resonance imaging. PET = positron emission tomography.

Table 6.3 Baseline clinical characteristics of study participants included in the *in vivo* ^{18}F-flutemetamol MRI-PET study

	All participants (n=20)	
Age (years); median (IQR)	71	(62-74)
Sex		
Female	6	(30)
Male	14	(70)
Co-morbidities		
Hypertension	8	(40)
Ischaemic stroke	3	(15)
Transient ischaemic attack	0	(0)
Dementia	0	(0)
Diabetes	2	(10)
Atrial fibrillation	1	(5)
Myocardial infarction	1	(5)
Hyperlipidaemia	1	(5)
Smoking status		
Current	0	(0)
Ex-smoker	14	(70)
Never	6	(30)
Pre-ICH modified Rankin scale; median (IQR)	0	(0-0)
Medications on admission		
Antiplatelet drug(s)	4	(20)
Anticoagulant drug(s)	1	(5)
Antihypertensive drug(s)	6	(30)
Admission GCS; median (IQR)	15	(14-15)
MMSE; median (IQR)	30	(29-30)
MOCA; median (IQR)	28	(26-29)
ACEIII; median (IQR)	96	(94-98)
Time between ICH and cognitive assessment (days); median (IQR)	245	(185-316)
Time between ICH and MRI-PET scan (days); median (IQR)	305	(240-350)
Time between cognitive assessment and MRI-PET scan (days); median (IQR)	41	(11-54)

ACE = Addenbrooke's cognitive examination. GCS = Glasgow coma scale. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. MMSE = mini mental state examination. MoCA = Montreal cognitive assessment. MRI = Magnetic resonance imaging. PET = Positron emission tomography.

Table 6.4 Modified Boston criteria MRI features in the *in vivo* ^{18}F-flutemetamol MRI-PET study participants

ICH	Multiple ICH	CMB count		Cortical superficial siderosis				Modified
location		Lobar	Non lobar	Presence	Location	Extent	Number of sulci	**Boston criteria**
Deep	No	0	3	No				
Deep	No	0	0	No				
Deep	No	0	0	No				
Deep	No	0	0	No				
Deep	No	0	0	No				
Deep	No	0	0	No				No CAA
Deep	No	0	0	No				
Deep	No	0	0	No				
Deep	Yes (deep)	0	0	No				
Deep	No	4	3	No				
Deep	No	0	0	No				
Lobar	No	14	1	Yes	Adjacent to ICH	Focal	1	
Lobar	No	0	0	No				Possible CAA
Lobar	No	102	0	Yes	Adjacent & distant to ICH	Diffuse	10	
Lobar	No	0	0	Yes	Adjacent & distant to ICH	Diffuse	10	
Lobar	No	0	0	Yes	Adjacent to ICH	Focal	1	
Lobar	No	0	0	Yes	Adjacent to ICH	Focal	1	Probable CAA
Lobar	No	9	0	Yes	Adjacent & distant to ICH	Focal	3	
Lobar	No	241	0	Yes	Adjacent & distant to ICH	Diffuse	4	
Lobar	Yes (lobar)	108	0	No				

CAA = cerebral amyloid angiopathy. CMB = cerebral microbleed. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. MRI = Magnetic resonance imaging. PET = Positron emission tomography.

Table 6.5 Baseline clinical features and cognitive assessment in CAA-associated versus non-CAA-associated ICH participants classified by the MRI-based modified Boston criteria reference standard

	Not CAA-associated ICH (n=13)		CAA-associated ICH (n=7)		p value
Age (years); median (IQR)	69	(60-72)	73	(71-77)	0.219
Sex*					
Female	3	(23)	3	(57)	0.612
Male	10	(77)	4	(43)	
Co-morbidities*					
Hypertension	6	(46)	2	(29)	0.642
Ischaemic stroke	2	(15)	1	(14)	1.000
Transient ischaemic attack	0	(0)	0	(0)	N/A
Dementia	0	(0)	0	(0)	N/A
Diabetes	1	(8)	1	(14)	1.000
Atrial fibrillation	0	(0)	1	(14)	0.350
Myocardial infarction	0	(0)	1	(14)	0.350
Hyperlipidaemia	0	(0)	1	(14)	0.350
Smoking status*					
Current	0	(0)	0	(0)	1.000
Ex-smoker	9	(69)	5	(71)	
Never	4	(31)	2	(29)	
Pre-ICH modified Rankin scale; median (IQR)	0	(0-0)	0	(0-0)	0.648
Medications on admission*					
Antiplatelet drug(s)	2	(15)	2	(29)	0.587
Anticoagulant drug(s)	0	(0)	1	(14)	0.350
Antihypertensive drug(s)	3	(23)	3	(43)	0.613
Admission GCS; median (IQR)	15	(15-15)	14	(14-15)	0.086
MMSE; median (IQR)	30	(29-30)	30	(30-30)	0.850
MoCA; median (IQR)	28	(27-28)	26	(25-26)	0.064
ACEIII; median (IQR)	97	(94-98)	95	(93-95)	0.139
Time between ICH and cognitive assessment (days); median (IQR)	257	(209-318)	209	(181-242)	0.245
Time between ICH and MRI-PET scan (days); median (IQR)	304	(247-345)	305	(232-345)	0.782
Time between cognitive assessment and MRI-PET scan (days); median (IQR)	30	(0-51)	49	(30-105)	0.088

* Fisher's exact test. ACE = Addenbrooke's cognitive examination. CAA = cerebral amyloid angiopathy. GCS = Glasgow coma scale. ICH = intracerebral haemorrhage. MMSE = mini mental state examination. MoCA = Montreal cognitive assessment. MRI = Magnetic resonance imaging. PET = Positron emission tomography.

The time between ICH and MRI-PET scan was similar between CAA-associated and non-CAA-associated ICH groups (median 305 days, IQR 247-345, versus 304 days, IQR 232-345 respectively). All 20 participants tolerated the full MRI-PET scan. There were no reported adverse effects.

Distribution of disease severity between CAA-associated and non-CAA-associated ICH groups

The CAA-associated ICH group had significantly more frequent cortical superficial siderosis and a higher number of lobar microbleeds than the non-CAA-associated ICH group as expected (Table 6.6). There was no difference in the presence or number of deep or cerebellar microbleeds, or the severity of WMH, atrophy or enlarged perivascular spaces between the groups. The median MRI CAA SVD burden score was higher in the CAA-associated ICH group.

6.4.2.2 Qualitative flutemetamol PET assessment

Inter-rater agreement

Inter-rater agreement of visual evaluation of the flutemetamol PET scans was almost perfect for overall positivity (κ 0.90, Table 6.7). Agreement for standard regions was substantial to perfect for all regions except the right parietal lobe, where there was moderate agreement. Agreement for the occipital lobe, which is not part of the standard assessment, was the poorest (κ 0.41 to 0.49).

Index test versus reference standard

Table 6.8 shows the consensus visual classification of flutemetamol PET scans (index test) against the modified Boston criteria classification (reference standard). Six of the seven probable CAA cases had a positive flutemetamol PET scan resulting in a sensitivity of 86% (95% CI 42% to 99%, Figure 6.8 and Figure 6.9). Ten of the 13 participants classified as no CAA or possible CAA had a negative flutemetamol PET scan, resulting in a specificity of 77% (95% CI 46 to 94%, Figure 6.10 and Figure 6.11, Table 6.9).

The clinical and imaging features of the one false negative and three false positive cases are summarised in Figure 6.12 and Figure 6.13 to Figure 6.15 respectively.

Table 6.6 MRI characteristics in participants classified as CAA-associated ICH versus non-CAA-associated ICH by the MRI-based modified Boston criteria reference standard

	Not CAA-associated ICH (n=13)	CAA-associated ICH (n=7)	P value
ICH location*			
Lobar	2 (15)	7 (100)	<0.001
Deep	11 (85)	0 (0)	
ICH epicentre			
Basal ganglia	8 (62)	0 (0)	
Thalamus	3 (23)	0 (0)	
Frontal Lobe	1 (8)	3 (43)	
Temporal Lobe	0 (0	2 (29)	
Parietal Lobe	0 (0)	0 (0)	
Occipital Lobe	1 (8)	2 (29)	
Periventricular Fazekas score; median (IQR)	3 (2-4)	4 (2-4)	0.507
Deep Fazekas score; median (IQR)	2 (2-3)	3 (2-3)	0.614
Central atrophy; median (IQR)	3 (2-3)	3 (3-5)	0.116
Cortical atrophy; median (IQR)	1 (1-1)	1 (1-3)	0.144
Basal ganglia PVS; median (IQR)	1 (1-2)	1 (1-1)	0.276
Centrum semiovale PVS; median (IQR)	3 (2-3)	2 (2-3)	0.413
Cortical superficial siderosis*	1 (8)	6 (86)	0.001
Any lobar CMB*	2 (15)	4 (57)	0.122
Lobar CMBs; median (IQR)	0 (0-0)	9 (0-104)	0.032
Any deep CMB*	3 (23)	0 (0)	0.521
Deep CMBs; median (IQR)	0 (0-0)	0 (0-0)	0.180
Any cerebellar CMB*	0 (0)	1 (14)	0.350
Cerebellar CMBs; median (IQR)	0 (0-0)	0 (0-0)	0.173
Any brainstem CMB*	0 (0)	0 (0)	N/A
Brainstem CMBs; median (IQR)	0 (0-0)	0 (0-0)	N/A
Any CMB*	3 (25)	4 (57)	0.326
Total CMBs; median (IQR)	0 (0-1)	9 (0-105)	0.071
MRI SVD burden score; median (IQR)	2 (1-4)	3 (3-3)	0.683
MRI CAA SVD burden score; median (IQR)	2 (1-2)	3 (3-6)	0.003

* Fisher's exact test. CAA = cerebral amyloid angiopathy. CMB = cerebral microbleed. ICH = intracerebral haemorrhage. MRI = Magnetic resonance imaging. PET = Positron emission tomography. PVS = perivascular space. SVD = small vessel disease.

Table 6.7 Inter-rater agreement for visual assessment of ^{18}F-flutemetamol PET scans

Region	Frequency positive, n (%)		Inter-rater agreement, κ (95% CI)
	Rater 1	Rater 2	
Right frontal pole	7 (35)	9 (45)	0.79 (0.70-0.89)
Left frontal pole	7 (35)	8 (40)	0.89 (0.82-0.96)
Right posterior cingulate & precuneus	8 (40)	8 (40)	1.00 (1.00-1.00)
Left posterior cingulate & precuneus	8 (40)	8 (40)	1.00 (1.00-1.00)
Right lateral temporal lobe	7 (35)	6 (30)	0.66 (0.54-0.78)
Left lateral temporal lobe	7 (35)	6 (30)	0.89 (0.81-0.96)
Right parietal lobe	7 (35)	3 (15)	0.49 (0.36-0.63)
Left parietal lobe	7 (35)	4 (20)	0.63 (0.51-0.76)
Right striatum	8 (40)	7 (35)	0.89 (0.82-0.96)
Left striatum	8 (40)	7 (35)	0.89 (0.82-0.96)
Right occipital	6 (30)	2 (10)	0.41 (0.27-0.56)
Left occipital	7 (35)	3 (15)	0.49 (0.36-0.63)
Overall	8 (40)	9 (45)	0.90 (0.70-1.00)

PET = positron emission tomography

Table 6.8 Cross-tabulations of the consensus visual ^{18}F-flutemetamol PET scan classification against the MRI-based modified Boston criteria

Flutemetamol visual classification (Index test)	Modified Boston criteria (Reference standard)		
	Probable CAA	No/possible CAA	Total
Positive	6	3	9
Negative	1	10	11
Total	7	13	20

MRI = magnetic resonance imaging. PET = positron emission tomography.

Table 6.9 Diagnostic test accuracy statistics for the consensus visual ^{18}F-flutemetamol PET scan classification against the MRI-based modified Boston criteria

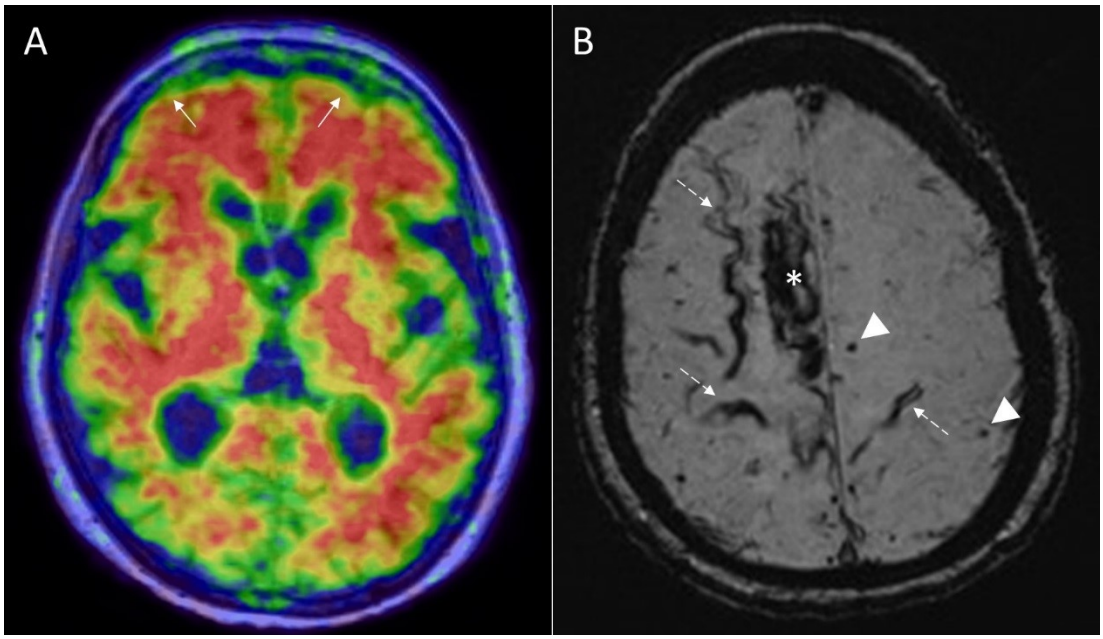
	Flutemetamol visual classification	
Sensitivity	86	(42-99)
Specificity	77	(46-94)
Positive likelihood ratio	3.7	(1.3-10.5)
Negative likelihood ratio	0.2	(0.0-1.2)
Positive predictive value	67	(31-91)
Negative predictive value	91	(57-100)

MRI = magnetic resonance imaging. PET = positron emission tomography.

Figure 6.8 True positive ^{18}F-flutemetamol PET scan versus the modified Boston criteria

A. Axial ^{18}F-flutemetamol PET image showing loss of the normal sulcal pattern and a sharp intensity gradient from grey matter to cerebrospinal fluid in the frontal pole (arrows).

B. Axial SWI showing a lobar ICH centred in the right frontal lobe (asterisk), diffuse cortical superficial siderosis (dashed arrows) and multiple lobar microbleeds (arrowheads). Classified as probable CAA on the modified Boston criteria.



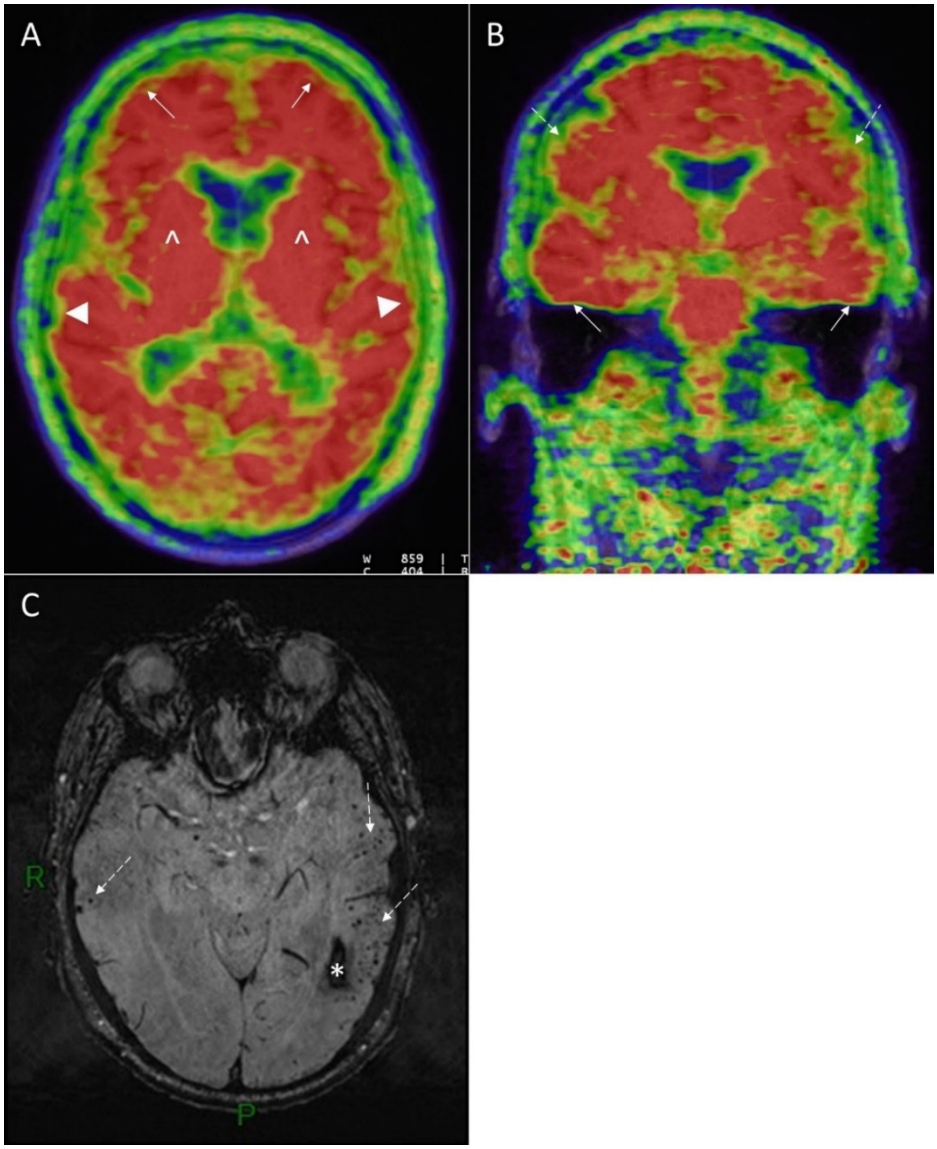
CAA = cerebral amyloid angiopathy. MRI = magnetic resonance imaging. PET = positron emission tomography. SWI = susceptibility-weighted imaging.

Figure 6.9 True positive ^{18}F-flutemetamol PET scan versus the modified Boston criteria

A. Axial 18F-flutemetamol PET image showing loss of the normal sulcal pattern and a sharp intensity gradient from grey matter to cerebrospinal fluid in the frontal pole (arrows) and lateral temporal lobes (arrowheads) plus loss of the normal strial gap (chevrons).

B. Coronal ^{18}F-flutemetamol image PET showing loss of the normal sulcal pattern and a sharp intensity gradient from grey matter to cerebrospinal fluid in the parietal (dashed arrows) and lateral temporal lobes (arrows).

C. Axial SWI showing a lobar ICH centred in the left temporal lobe (asterisk) and multiple lobar microbleeds (dashed arrows). Classified as probable CAA on the modified Boston criteria.

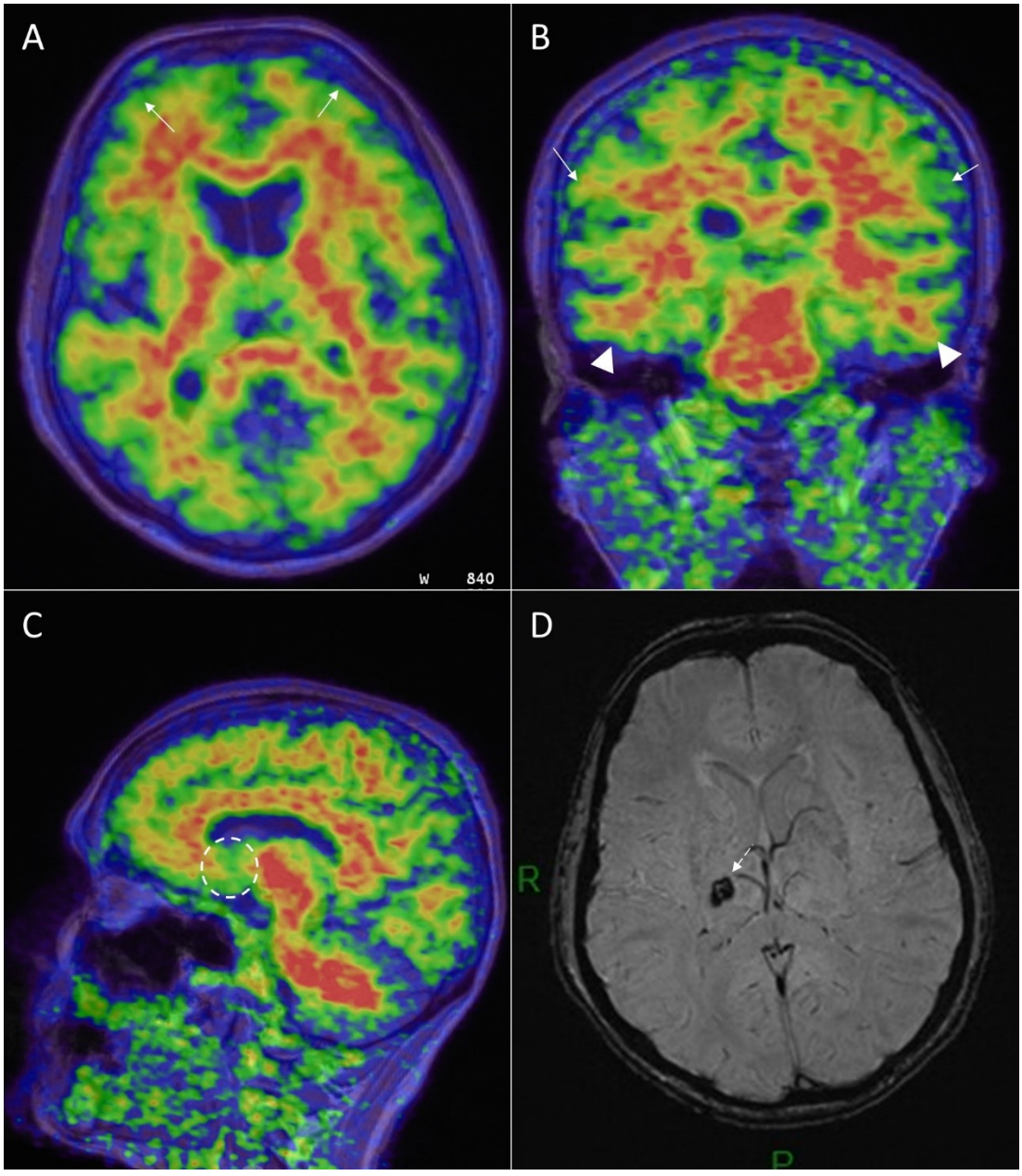


CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage. MRI = magnetic resonance imaging. PET = positron emission tomography. SWI = susceptibility-weighted imaging.

Figure 6.10 True negative ^{18}F-flutemetamol PET scan versus the modified Boston criteria

A - C. Axial, coronal and sagittal ^{18}F-flutemetamol PET images showing the normal sulcal pattern and a gradual intensity gradient from grey matter to cerebrospinal fluid in the frontal pole (arrows), parietal lobe (arrows) and lateral temporal lobes (arrowheads). The normal striatal gap is present (dotted circle).

D. Axial SWI showing a single deep ICH centred in the right thalamus (dashed arrow). There were no microbleeds or cortical superficial siderosis. Classified as no CAA on the modified Boston criteria.

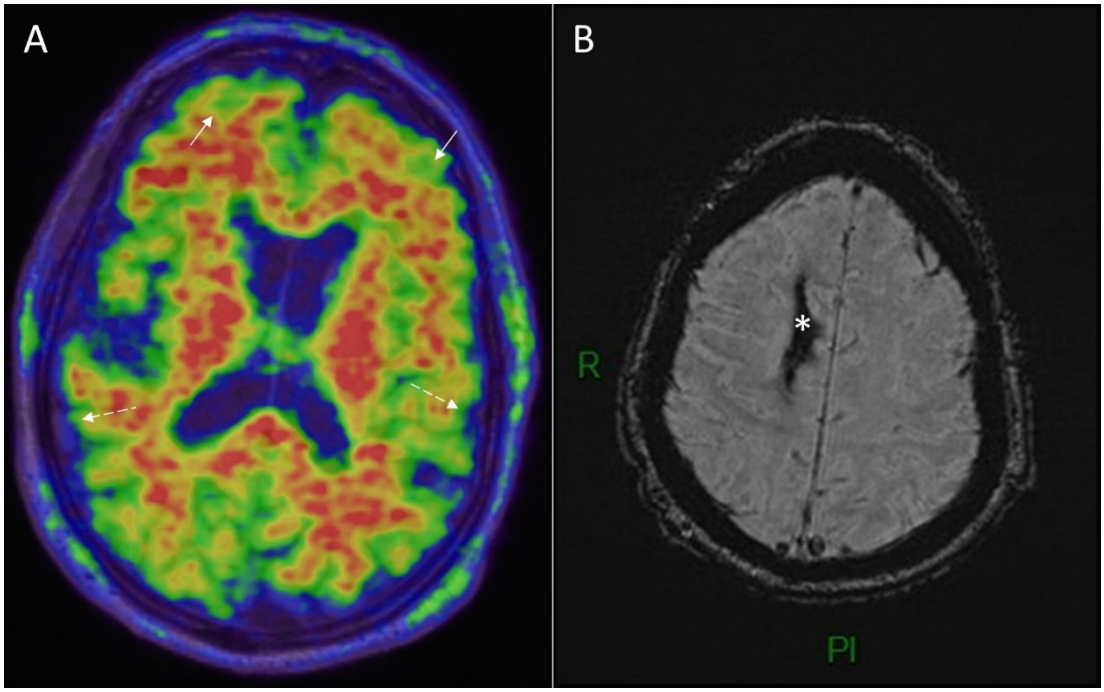


CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage. MRI = magnetic resonance imaging. PET = positron emission tomography. SWI = susceptibility-weighted imaging.

Figure 6.11 True negative ^{18}F-flutemetamol PET scan versus the modified Boston criteria

A. Axial ^{18}F-flutemetamol PET image showing the normal sulcal pattern and a gradual intensity gradient from grey matter to cerebrospinal fluid in the frontal pole (arrows) and lateral temporal lobes (dashed arrows).

B. Axial SWI showing a single lobar ICH centred in the right frontal lobe (asterisk). There were no microbleeds or cortical superficial siderosis. Classified as possible CAA on the modified Boston criteria.



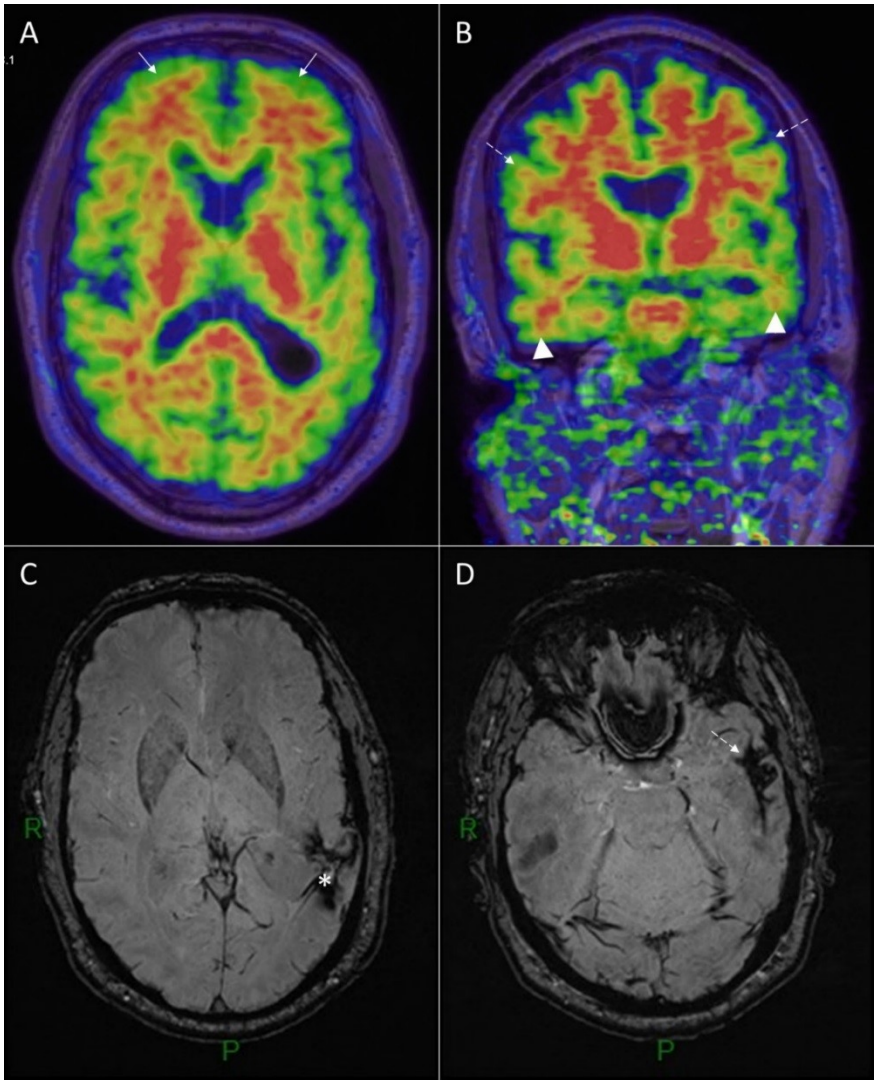
CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage. MRI = magnetic resonance imaging. PET = positron emission tomography. SWI = susceptibility-weighted imaging

Figure 6.12 False negative ^{18}F-flutemetamol PET scan versus the modified Boston criteria

55 year-old male with first-ever symptomatic ICH. No pre-ICH history of hypertension or anthithrombotic drug use. MMSE 30/30, MoCA 29/30, ACE III 93/100.

A and B. Axial and coronal ^{18}F-flutemetamol PET images showing the normal sulcal pattern and a gradual intensity gradient from grey matter to cerebrospinal fluid in the frontal pole (arrows), parietal lobe (dashed arrows) and lateral temporal lobes (arrowheads).

C and D. Axial SWI images showing a single lobar ICH centred in the left temporal lobe (asterisk) and focal cortical superficial siderosis but no microbleeds. Classified as probable CAA on the modified Boston criteria.



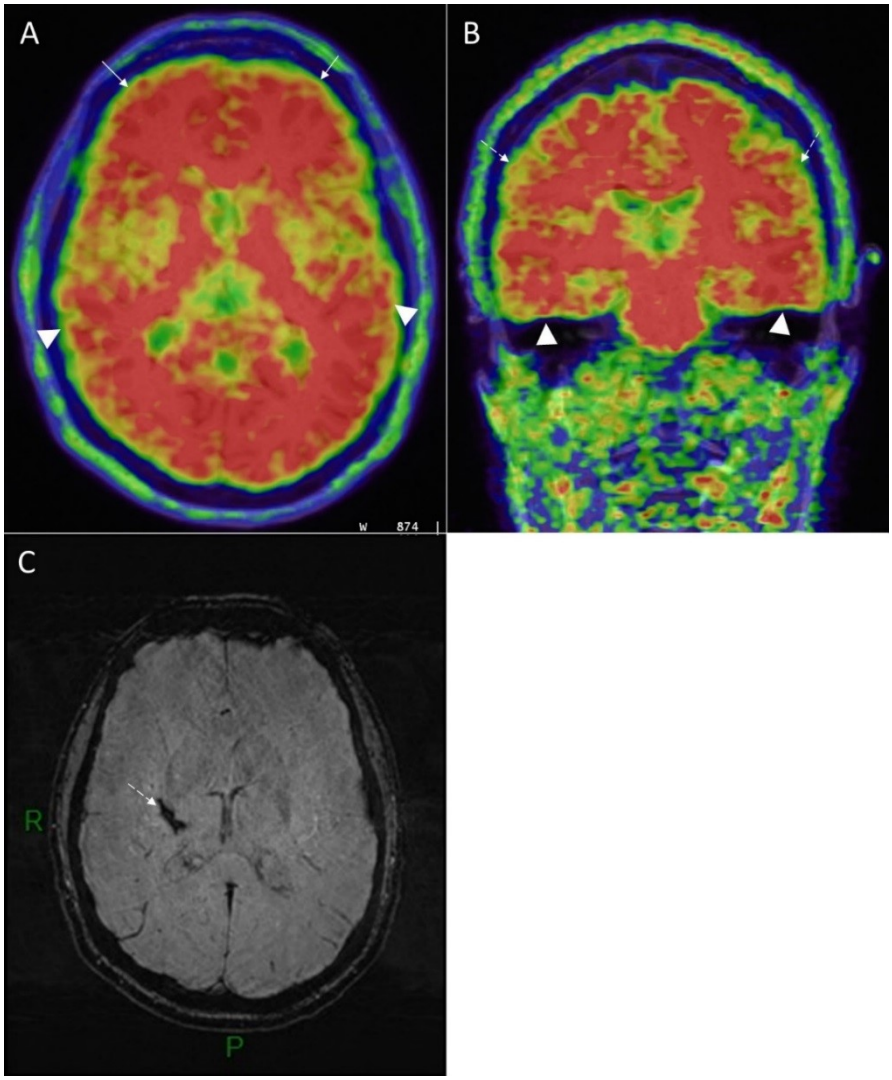
ACE = Addenbrooke's cognitive examination. CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage. MoCA = Montreal cognitive assessment. MMSE = mini mental state exam. MRI = magnetic resonance imaging. PET = positron emission tomography. SWI = susceptibility-weighted imaging.

Figure 6.13 False positive ^{18}F-flutemetamol PET scan versus the modified Boston criteria

53 year-old female with first-ever symptomatic ICH. Pre-ICH history of hypertension. No pre-ICH anthithrombotic drug use. MMSE 30/30, MoCA 29/30, ACE III 100/100.

A. Axial and coronal ^{18}F-flutemetamol PET images showing loss of the normal sulcal pattern and a sharp intensity gradient from grey matter to cerebrospinal fluid in the frontal pole (arrows), lateral temporal lobes (arrowheads) and the parietal lobes (dashed arrows).

C. Axial SWI showing a single deep ICH in the right basal ganglia (dashed arrow). No microbleeds or cortical superficial siderosis. Classified as no CAA on the modified Boston criteria.



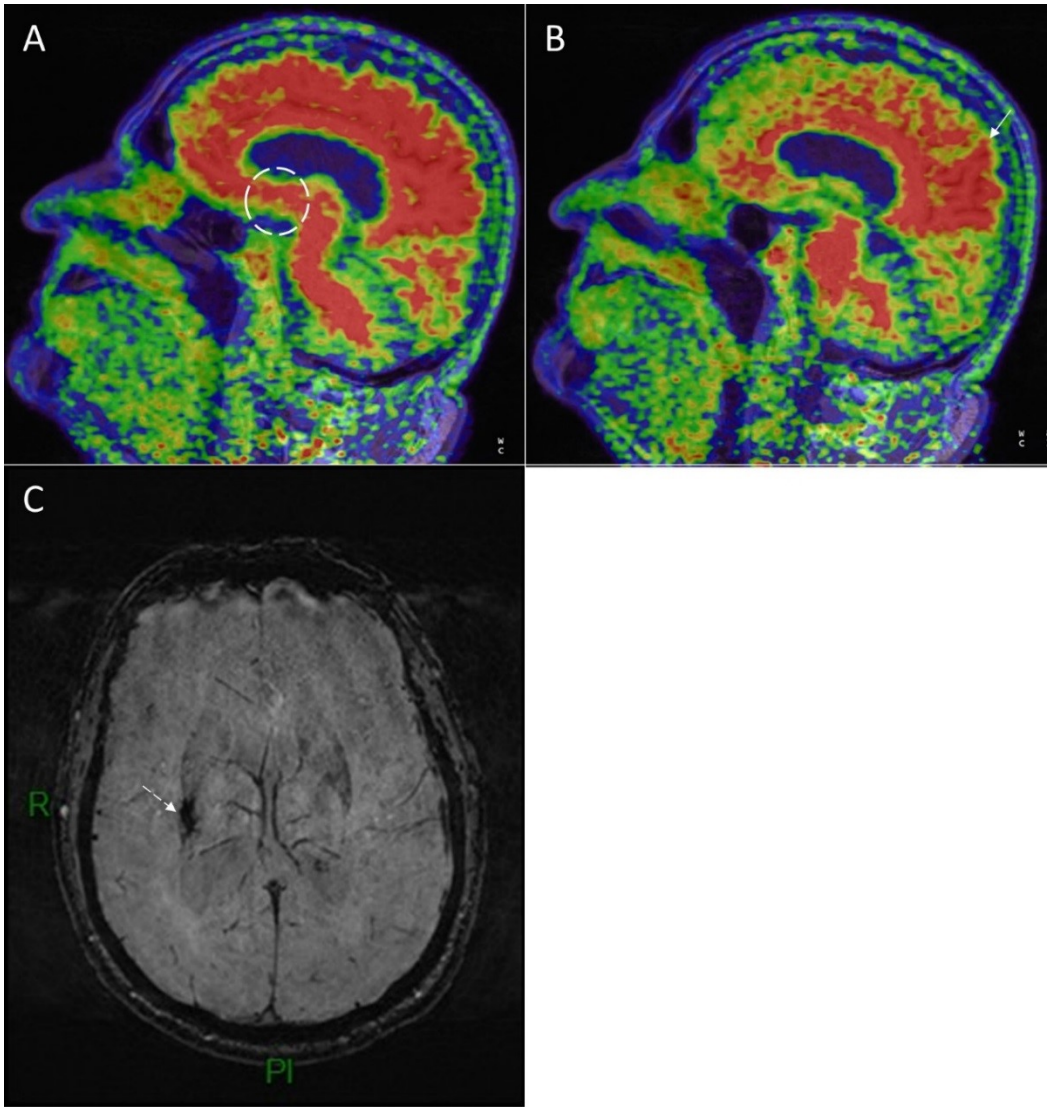
ACE = Addenbrooke's cognitive examination. CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage. MoCA = Montreal cognitive assessment. MMSE = mini mental state exam. MRI = magnetic resonance imaging. PET = positron emission tomography. SWI = susceptibility-weighted imaging.

Figure 6.14 False positive ^{18}F-flutemetamol PET scan versus the modified Boston criteria

75 year-old male with first-ever symptomatic ICH. Pre-ICH history of hypertension. No pre-ICH anthithrombotic drug use. MMSE 29/30, MoCA 30/30, ACE III 97/100.

A and B. Sagittal ^{18}F-flutemetamol PET images showing loss of the normal striatal gap (dashed circle) and the presence of cortical uptake in the precuneus (arrow).

C. Axial SWI showing a single deep ICH in the right basal ganglia (dashed arrow). No microbleeds or cortical superficial siderosis. Classified as no CAA on the modified Boston criteria.



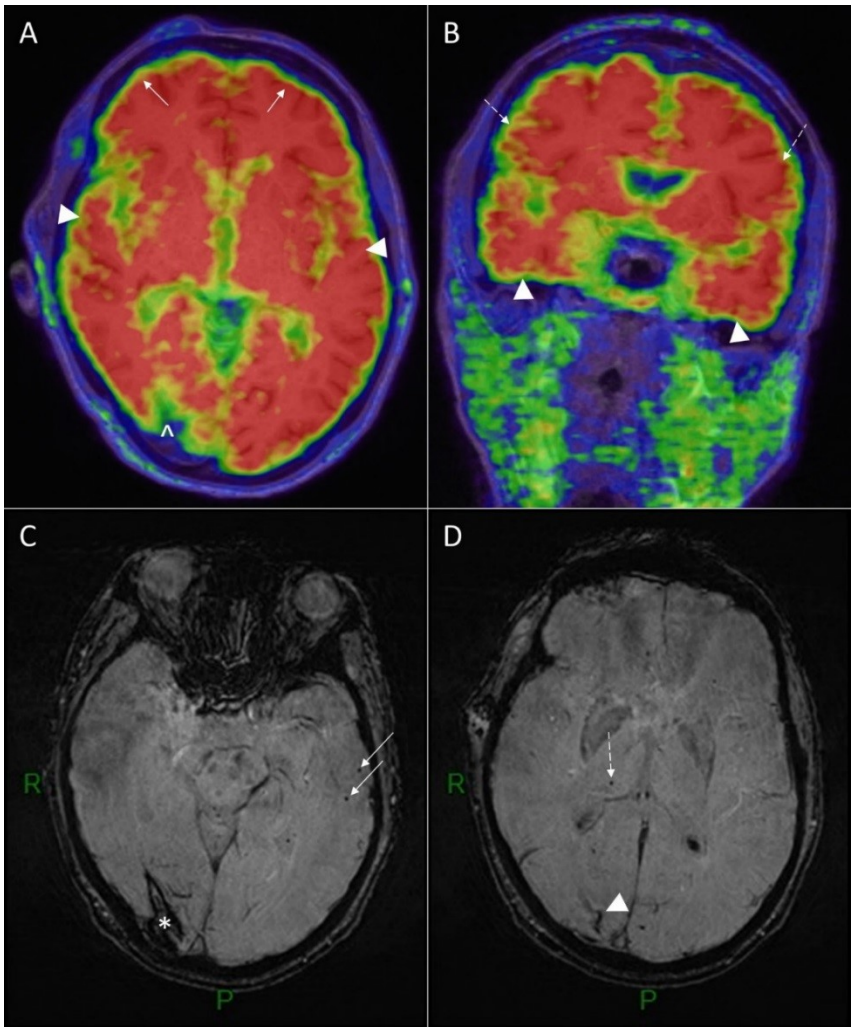
ACE = Addenbrooke's cognitive examination. CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage. MoCA = Montreal cognitive assessment. MMSE = mini mental state exam. MRI = magnetic resonance imaging. PET = positron emission tomography. SWI = susceptibility-weighted imaging.

Figure 6.15 False positive ^{18}F-flutemetamol PET scan versus the modified Boston criteria

80 year-old male with first-ever symptomatic ICH. Pre-ICH history of hypertension and antiplatelet drug use. MMSE 30/30, MoCA 29/30, ACE III 97/100.

A. Axial and coronal ^{18}F-flutemetamol PET images showing loss of the normal sulcal pattern and a sharp intensity gradient from grey matter to cerebrospinal fluid in the frontal pole (arrows), lateral temporal lobes (arrowheads) and the parietal (dashed arrows) lobes.

C and D. Axial SWI showing a single lobar ICH in the right occipital lobe (asterisk). Multiple lobar microbleeds (arrows) and focal cortical superficial siderosis (arrowhead). A single deep microbleed is present in the right thalamus (dashed arrow). Classified as no CAA on the modified Boston criteria.



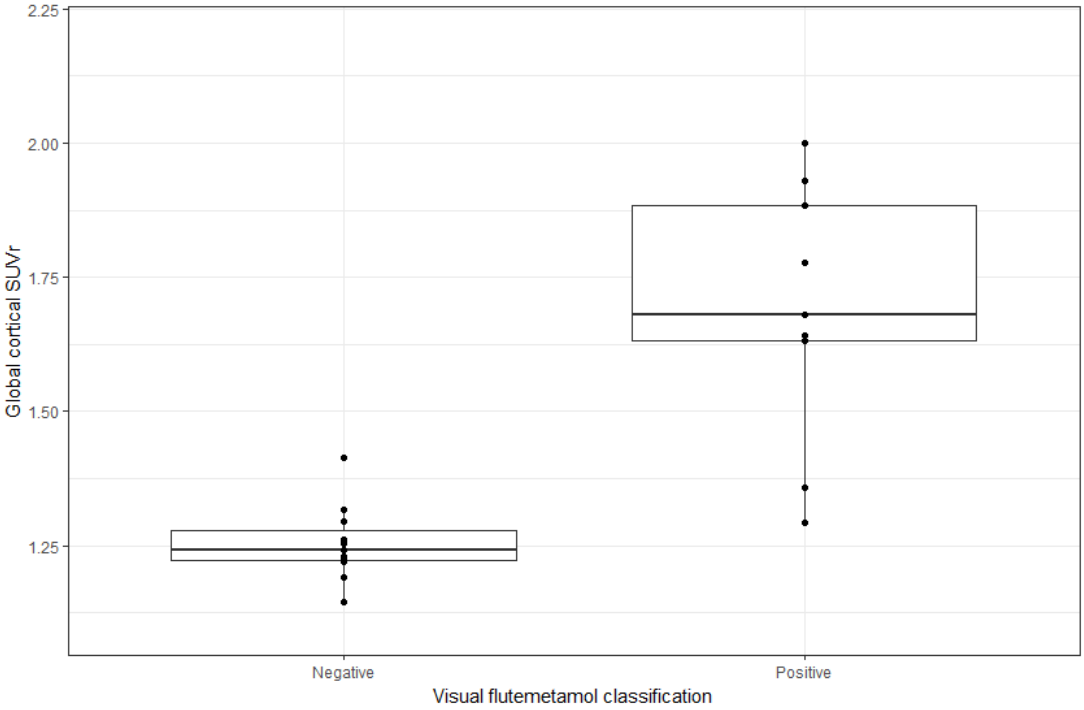
ACE = Addenbrooke's cognitive examination. CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage. MoCA = Montreal cognitive assessment. MMSE = mini mental state exam. MRI = magnetic resonance imaging. PET = positron emission tomography. SWI = susceptibility-weighted imaging.

6.4.2.3 Quantitative flutemetamol PET analysis

Comparison of qualitative and quantitative analyses

We classified nine out of 20 flutemetamol scans as positive by consensus visual assessment. The global cortical SUVr in these cases (median 1.68 [IQR 1.63-1.88]) was significantly higher than those classified as negative (median 1.24 [IQR 1.22-1.28], p=0.0001). However, the global cortical SUVr overlapped between two positive and three negative cases (SUVr 1.29 to 1.41) (Figure 6.16).

Figure 6.16 Boxplots showing ^{18}F-flutemetamol consensus visual classification against global cortical SUVr

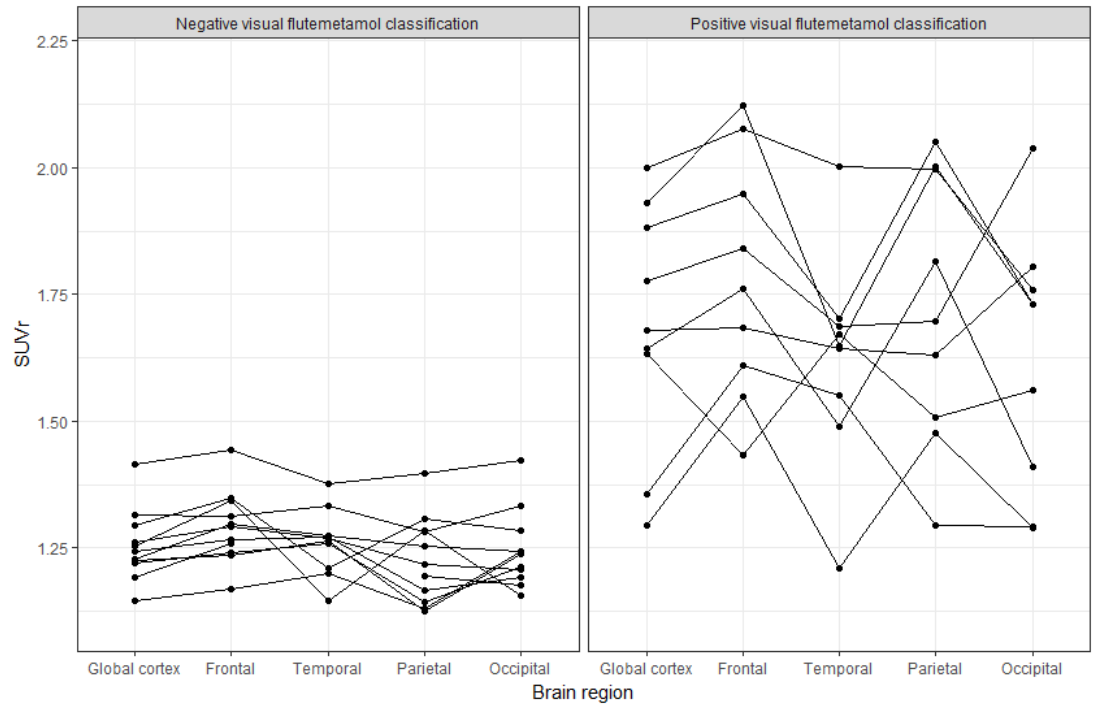


SUVr = standardised uptake value ratio.

Flutemetamol scans are classified as positive on visual assessment if at least one cortical region is positive. Therefore, I compared the SUVr of individual cortical VOIs between the nine visual positive and the 11 visual negative scans (Figure 6.17). The maximum cortical regional SUVr of the scans classified as positive by visual rating (median 1.88 [IQR 1.78-2.07; range

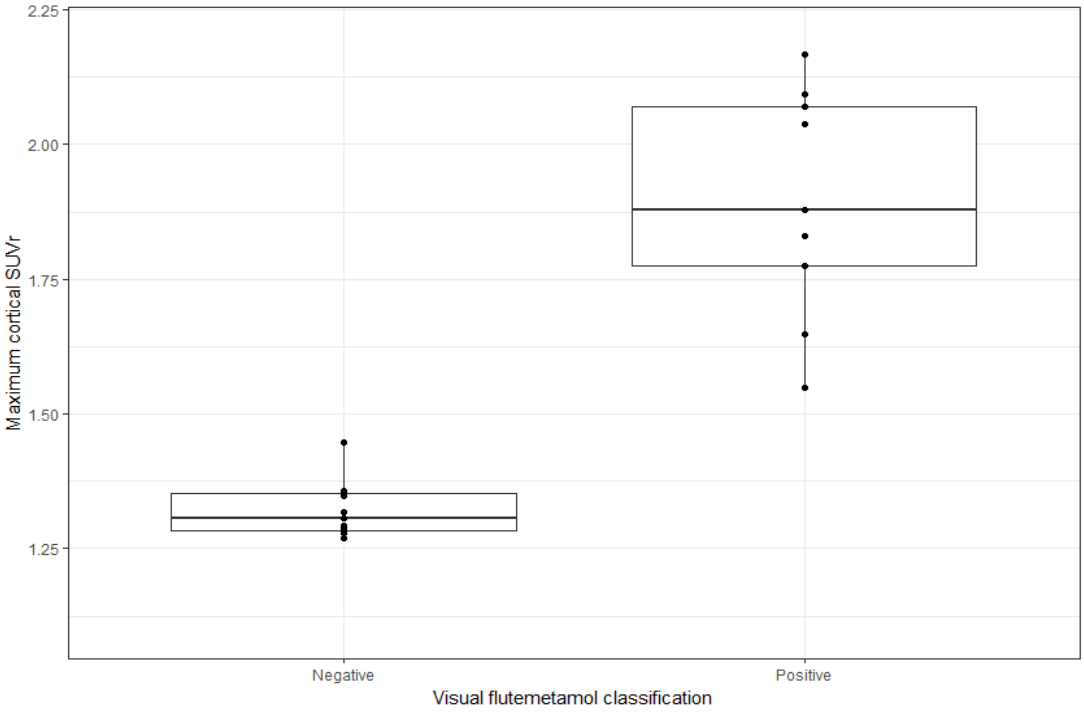
1.55-2.17]) was significantly higher than those classified as negative (median 1.31 [IQR 1.28-1.35; range 1.27-1.45], p<0.0001), without any overlap (Figure 6.18).

Figure 6.17 Line plots showing global and regional cortical ^{18}F-flutemetamol SUVr stratified by consensus visual flutemetamol classification



SUVr = standardised uptake value ratio.

Figure 6.18 Boxplots showing ^{18}F-flutemetamol consensus visual classification against the maximum cortical SUVr

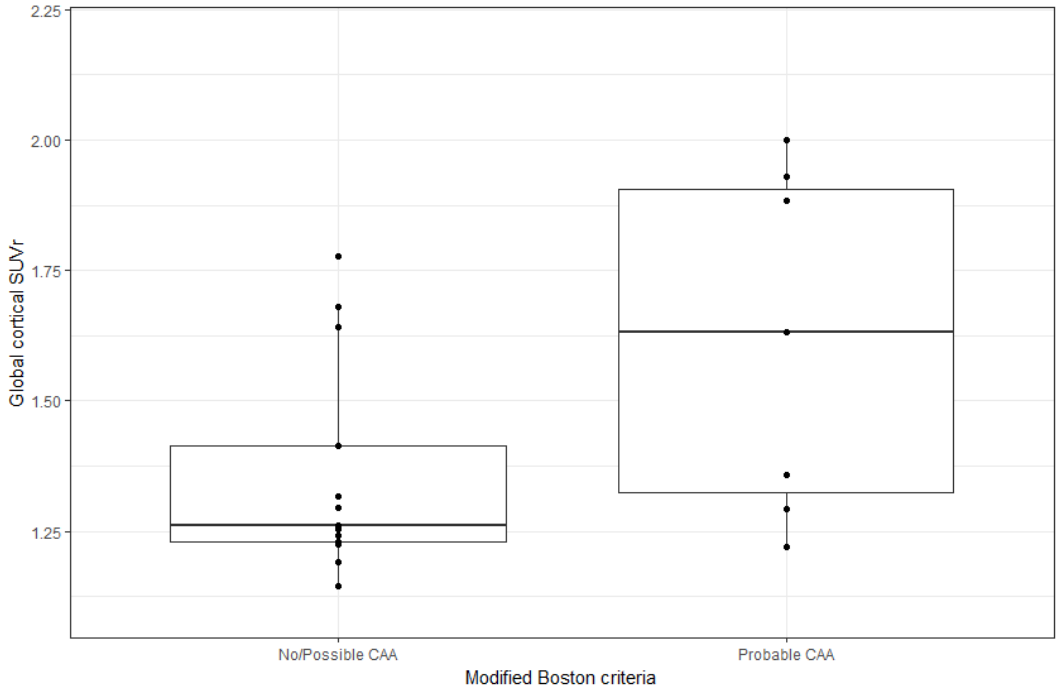


SUVr = standardised uptake value ratio.

Quantitative flutemetamol PET analysis associated with CAA-associated ICH versus non-CAA-associated ICH

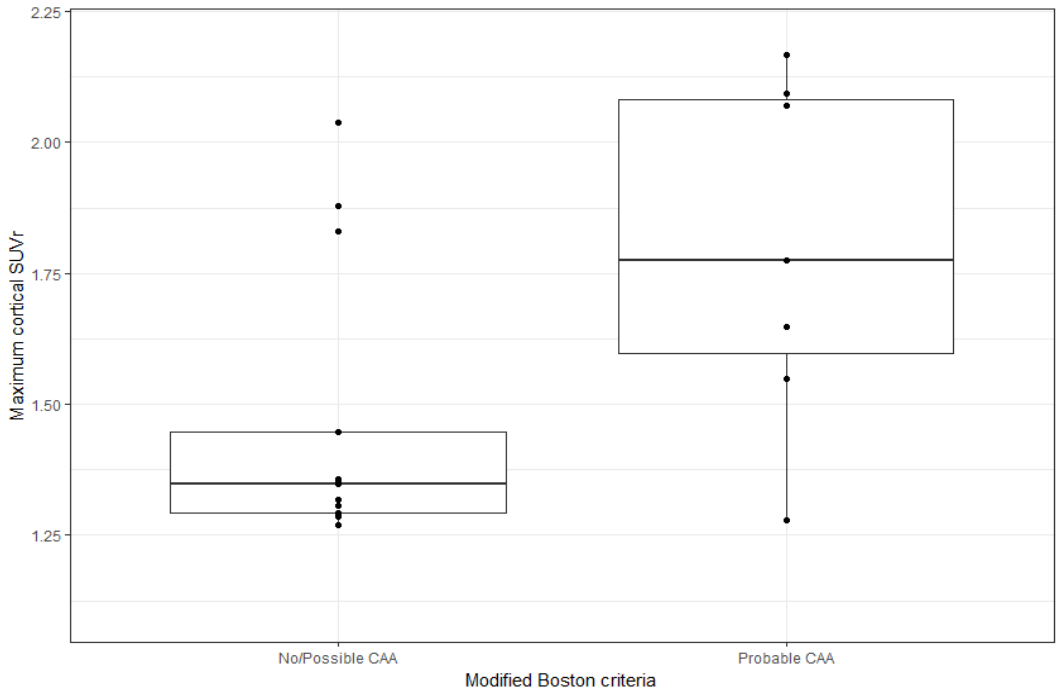
The median global cortical SUVr was higher in CAA-associated ICH than non-CAA-associated ICH, although this did not reach statistical significance (median 1.63 [IQR 1.33-1.91] versus 1.26 [IQR 1.23-1.41], p=0.097, Figure 6.19). The maximum regional cortical SUVr in CAA-associated ICH was significantly higher than the non-CAA-associated ICH group (median 1.78 [IQR 1.60-2.08] versus 1.35 [IQR 1.29-1.45] respectively, p = 0.046, Figure 6.20). The occipital/global cortex ratio was significantly lower in CAA-associated ICH compared with the non-CAA-associated ICH group (median 0.95 [IQR 0.91-0.98] versus 1.01 [IQR 0.96-1.01] respectively, p=0.037), while there was no difference in the frontal/global cortex ratio between CAA-associated ICH and non-CAA-associated ICH (median 1.04 [IQR 1.03-1.14] versus 1.02 [IQR 1.02-1.06] respectively, p=0.393).

Figure 6.19 Boxplots showing modified Boston criteria classification against global cortical ^{18}F-flutemetamol SUVr



SUVr = standardised uptake value ratio.

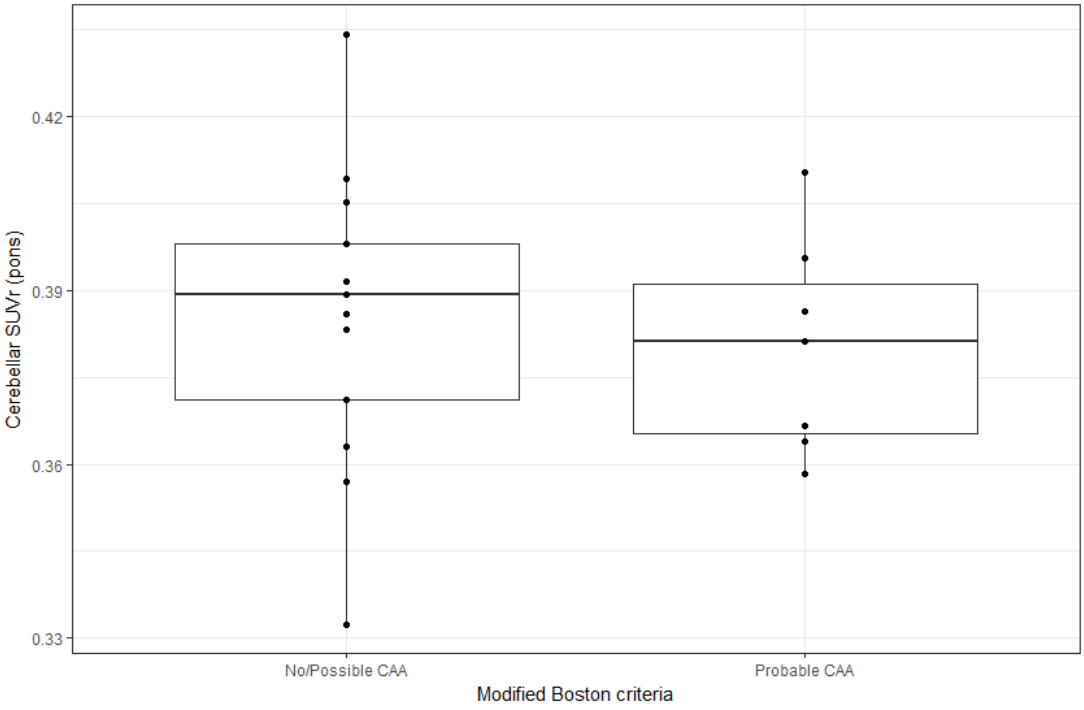
Figure 6.20 Boxplots showing modified Boston criteria classification against the maximum cortical SUVr



SUVr = standardised uptake value ratio.

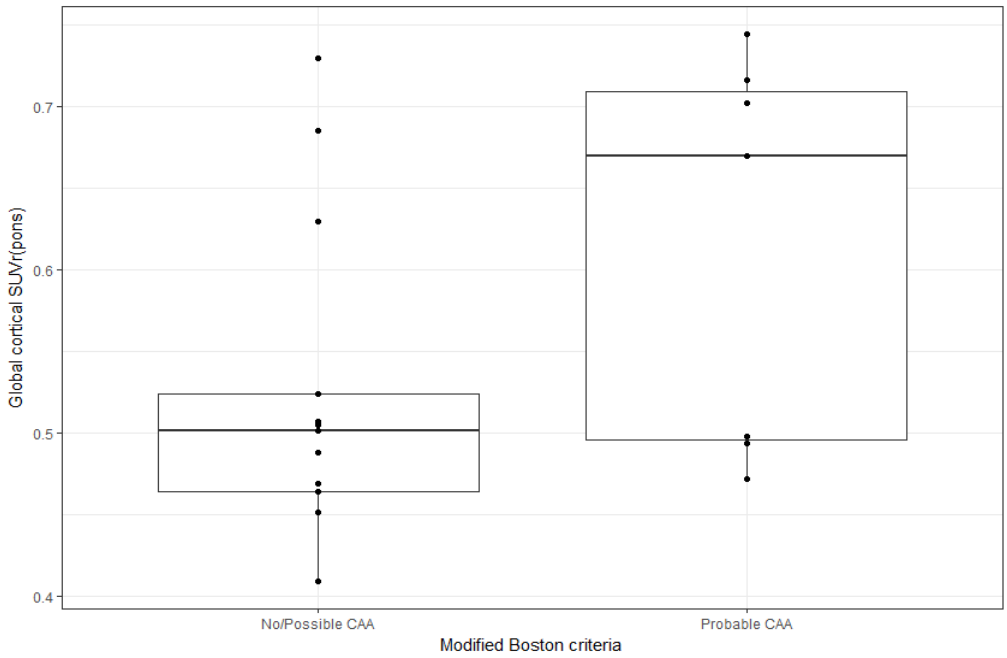
There was no difference in cerebellar SUVr using the pons as the reference region between the groups (CAA-associated ICH median 0.38 [IQR 0.37-0.39] versus non-CAA-associated ICH median 0.39 [IQR0.37-0.40], p=0.643) (Figure 6.21). Repeating the global and maximum cortical SUVr analyses with the pons as the reference region showed similar significance, direction, and magnitude to the analyses using the cerebellar cortex as the reference region (Figure 6.22 and Figure 6.23).

Figure 6.21 Boxplots showing the cerebellar SUVr using the pons as the reference standard stratified by the modified Boston criteria classification



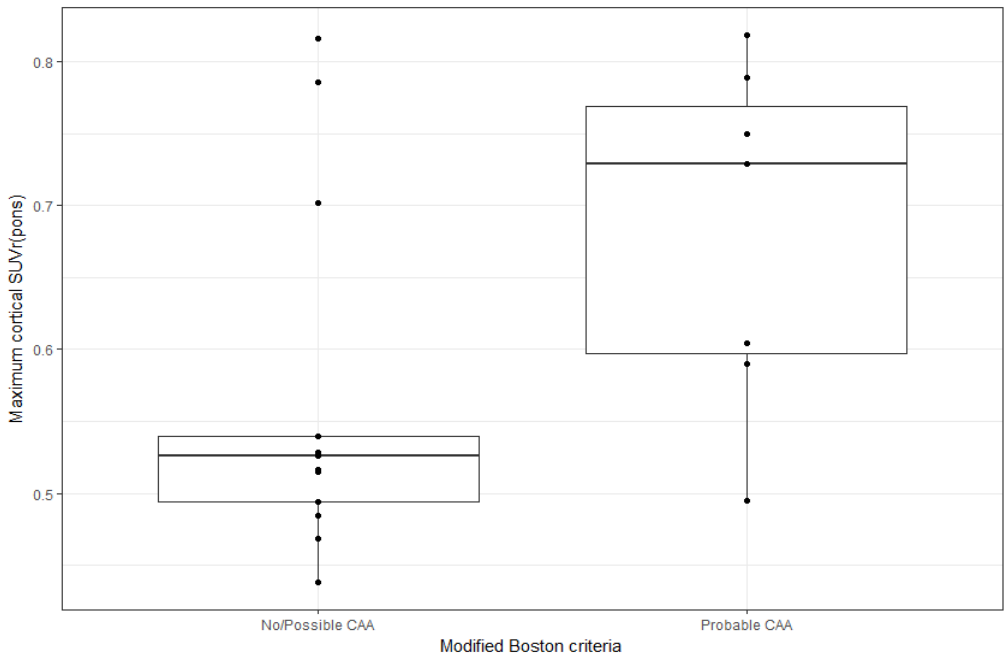
SUVr = standardised uptake value ratio.

Figure 6.22 Boxplots showing modified Boston criteria classification against global cortical ^{18}F-flutemetamol SUVr using the pons as the reference region



SUVr = standardised uptake value ratio.

Figure 6.23 Boxplots showing modified Boston criteria (reference standard) classification against the maximum cortical SUVr using the pons as the reference region



SUVr = standardised uptake value ratio.

6.5 Discussion

6.5.1 Main findings

- 6-CN-flutemetamol labels both perivascular (CAA) and non-vascular β-amyloid in *ex vivo* brain tissue.
- Overall visual assessment of flutemetamol scans between two trained observers was almost perfect (κ 0.90).
- Flutemetamol PET was positive by visual assessment in 6 out of 7 participants with probable CAA on the modified Boston criteria ("CAA-associated ICH"), and negative in 10 out of 13 participants with possible CAA or no CAA on the modified Boston criteria ("non-CAA-associated ICH"), resulting in 86% sensitivity and 77% specificity.
- Visual assessment of flutemetamol scans correlated with maximum cortical SUVr with no overlap between positive and negative visual classifications.
- Global cortical SUVr was higher in CAA-associated ICH versus non-CAA-associated ICH, although this difference did not reach statistical significance.
- Maximum cortical SUVr was significantly higher in CAA-associated ICH versus non-CAA-associated ICH, however there was overlap between the groups.

6.5.2 Strengths of the studies

6.5.2.1 Ex vivo 6-CN-flutemetamol study

I selected cases from the LINCHPIN brain bank to represent the extremes of non-vascular parenchymal and perivascular β-amyloid severity, based on systematic histopathological assessment using validated scales.[36, 219] We prepared and analysed all sections together, using the same reagents and parameters to minimise processing variability. We co-stained each section with markers for blood vessels (Col IV) and β-amyloid (6E10) to allow accurate localisation of 6-CN-flutemetamol labelling.

6.5.2.2 In vivo ^{18}F-flutemetamol MRI-PET studies

I performed and reported the ^{18}F-flutemetamol diagnostic accuracy study according to the STARD guidelines.[173] Important strengths are:

- I formulated the study questions and designed the study before performing the index test and reference standard.[175]
- I used prospective case ascertainment to minimise selection bias by inviting all potentially eligible patients to the study.[175, 287]
- I only included participants with first-ever ICH to provide a standard inception point.
- I used an appropriate control group with a similar distribution of vascular risk factors, cognition and MRI features of SVDs to the cases.
- I performed the index test and reference standard during a standard timeframe after the index ICH.
- All participants underwent the same index test and reference standard to avoid partial and differential verification biases.[174, 176]
- I minimised information bias by using a standard MRI-PET acquisition protocol for all participants, following manufacturer guidance for assessment of the index test, including completing the manufacturer's Electronic Training Programme before assessing PET scans,[308] using a validated scale to evaluate the reference standard[110] and masking assessors.[175]
- There were no missing data.
- I reported the flow of participants through the study, their baseline clinical and radiographic features and the distribution of disease severity in the study groups to illustrate selection bias.[175, 176]
- I used an image analysis protocol to standardise the quantitative PET analysis, including defining standard VOIs on the normalised PET images using automated anatomical labelling.

6.5.3 Limitations of the studies

6.5.3.1 Ex vivo 6-CN-flutemetamol study

I showed that 6-CN-flutemetamol labels both non-vascular parenchymal and perivascular β-amyloid when incubated with formalin fixed paraffin embedded section for two hours. However, this approach is clearly different to the mechanisms involved with *in vivo* ^{18}F-flutemetamol PET scanning. In particular, I was unable to assess the distribution of ^{18}F-flutemetamol from the vascular system through the blood-brain barrier into the brain parenchyma. However, previous studies have assessed the bio-distribution of ^{18}F-flutemetamol and demonstrated that it is rapidly distributed throughout the body, including the brain.[310] Also, the sample size I used for this exploratory study was small and the cases represented the extremes of non-vascular parenchymal and perivascular β-amyloid.

6.5.3.2 In vivo ^{18}F-flutemetamol MRI-PET studies

Despite using a prospective ascertainment approach, there is a substantial selection bias. Those included in the study tended to be relatively young, with little pre-ICH disability, small ICH volumes and high admission GCS compared with the community-based LATCH study of ICH (Table 3.3). This bias has implications for the generalisability of the study findings and the applicability of flutemetamol MRI-PET in clinical practice. However, studying patients with milder ICHs who may have less severe SVDs and who make a good functional recovery is clinically relevant, as this group have the most to gain from modifying the SVD process and preventing future haemorrhagic and vaso-occlusive events.

The sample size was small. Performing ^{18}F-flutemetamol PET-MR was difficult for several reasons. Firstly, the proportion of potentially eligible participants I included was low (20/198 = 10.1%). These findings are similar to a recent study assessing florbetapir PET in ICH, which included only 10.7% potentially eligible participants.[303] The other studies of amyloid PET in ICH did not describe the flow of participants.[252, 262, 299, 302, 304, 311] Over 60% of patients with ICH were excluded, mainly because they died

within six months (n=29), had pre-existing dementia (n=15) or a non-MRI compatible medical device (n=7). Over 40% of eligible participants were unable to tolerate MRI-PET scanning because they could not lie flat, were claustrophobic or not independently mobile. These reasons for exclusion are similar to the recent florbetapir PET study.[303]

Secondly, ^{18}F labelled PET tracers, such as flutemetamol, are produced on the day of the scan due to their 120-minute half-life. The production process has several steps, takes five to six hours and the final tracer has to meet strict quality control checks.[312] If there is a problem with flutemetamol production, the scan has to be postponed as there is insufficient time to remanufacture the tracer. During my study, I had to reschedule a total of 15 MRI-PET scans for seven participants due to problems with tracer production. This equates to a 57% (95% CI 41-72%) success rate for flutemetamol production.

My interim analysis includes less than half the pre-specified sample size and increases the risk of a type II error. The lack of power prevented me from adjusting the analyses for known confounders of amyloid PET uptake, such as age and cognitive impairment. Such analyses are particularly important given the slight differences in age and cognition between the CAA-associated ICH and non-CAA-associated ICH groups (Table 6.5).

The emission PET data should be corrected for spurious or missing events. In particular correction for the absorption or scatter of γ-rays by intervening tissue is important to allow accurate qualitative and quantitative analysis. A γ-ray transmission or low-dose CT scan are usually used to produce an attenuation map to correct for γ-ray attenuation. However, the small bore in hybrid MRI/PET systems cannot permit either of these approaches. There are several MRI-based attenuation correction approaches, such as template-based, atlas-based and direct segmentation-based and sequence-based, each with its advantages and disadvantages.[313, 314] I chose to use MRAC-UTE, a sequence-based approach for two reasons. In brain imaging, the bone and air-filled cavities are the most important “tissue” types for

attenuation correction.[313] UTE sequences were developed to differentiate tissues such as bone and air based on their very short T2 relaxation times.[315, 316] I chose not to use template-, atlas- or direct-segmentation methods as these are all based on tissue-dependent attenuation coefficients derived from reference datasets, which are unlikely to include participants with previous ICH. The participants in my study all had a previous ICH, which may distort the normal anatomy and alters the attenuation coefficient of brain parenchyma.

The input concentration of the tracer delivered to the tissue of interest should be estimated to perform quantitative PET analysis. Arterial blood sampling is the most accurate method but is invasive and not commonly performed in clinical PET studies.[317] Instead I used the cerebellar cortex as a tissue reference region, which is a common approach in the studies of amyloid-PET in ICH.[252, 262, 299, 300, 302-304] The ideal reference region is tissue with similar tracer kinetic properties to the target tissue (cerebral cortex) with negligible specific binding.[317] In Section 4.4.6 I demonstrated that the cerebellum is affected by CAA, especially meningeal CAA. The presence of CAA in cerebellar cortex could affect SUVr measures. However, I found similar results when I repeated the analyses using the pons as the reference region, and there was no difference in cerebellar $SUVr_{(pons)}$ between the CAA-associated ICH and non-CAA-associated ICH groups.

The ideal reference standard is systematic histopathological assessment for CAA and other SVDs, however, none of the participants had this. Instead, I used the MRI-based modified Boston criteria as the reference standard.[110] While the modified criteria are considered the *in vivo* clinical reference standard, they have moderate specificity in the development setting, and the sensitivity was less than 100%. The performance of the criteria in other settings is likely to be worse (Chapter 5). Therefore, their use as a reference standard in the study presents challenges for interpreting the diagnostic accuracy of flutemetamol PET. Are false negative or positive results due to misclassification by the index test or reference standard?

The single false negative flutemetamol case was a 55-year male who had no history of hypertension and did not take any antithrombotic drugs before his ICH. His cognition was normal after ICH. He was classified as probable CAA by the reference standard due to a single temporal macrohaemorrhage and focal cortical superficial siderosis affecting one sulcus, but no microbleeds. One false positive case was an 80-year-old male with a history of hypertension who took an antiplatelet agent before the index ICH. His cognition was normal. His MRI showed a single occipital macrohaemorrhage, focal cortical superficial siderosis, 14 lobar microbleeds. However, the presence of a single deep microbleed in the thalamus resulted in classification by the reference standard as non-CAA-associated ICH. The modified Boston criteria have a lower diagnostic certainty in these situations.[243] It is unclear whether the index test or reference standard wrongly classified this participant.

The two other false positive cases both had a history of hypertension, were not taking any antithrombotic drugs at the time of ICH and had normal cognition after-ICH. They both had a deep ICH affecting the lentiform nucleus without cortical superficial siderosis or microbleeds. Given the lack of association between deep ICH and CAA,[27, 28, 153] their positive flutemetamol PET results are likely to reflect the background rate of 9-35% flutemetamol positivity in cognitively normal older adults.[318, 319] This presumably indicates asymptomatic Alzheimer's disease or incidental CAA.

6.5.4 Comparison with other studies

6.5.4.1 Ex vivo 6-CN-flutemetamol study

Previous *in vitro* experiments have shown that flutemetamol binds to fibrillary β– amyloid in human Alzheimer brain homogenate assays and that 6-CN-flutemetamol binding correlates significantly with fibrillary β–amyloid in human autopsy brain tissue samples.[310] Clinical studies have shown that cortical uptake of ^{18}F-flutemetamol on PET correlates with the percent of biopsy specimen area staining for fibrillary β–amyloid in patients with normal pressure hydrocephalus.[320] A study of 106 end-of-life participants showed

that *in vivo* ^{18}F-flutemetamol PET can identify fibrillary β-amyloid identified on autopsy histopathology.[321]

The binding of Thioflavin-T derivatives, such as flutemetamol, is not specific to β-amyloid fibrils, and can also bind to insoluble β-amyloid such as CAA and diffuse plaques.[322, 323] No previous study has directly assessed flutemetamol binding to CAA in ICH. In the study of 106 end-of-life participants undergoing in vivo ^{18}F-flutemetamol PET and subsequent autopsy, three participants had CAA in the absence of Alzheimer's disease on histopathological assessment. However, two had a negative *in vivo* ^{18}F-flutemetamol PET, one of whom had minimal and focal CAA. The authors stated that no firm conclusion relating to the contribution of CAA to ^{18}F-flutemetamol PET uptake could be made due to the small numbers. However, they did show indirect evidence that CAA may contribute to cortical ^{18}F-flutemetamol retention in several cases with a positive ^{18}F-flutemetamol PET scan and borderline low levels of non-vascular β-amyloid but associated CAA.[321, 324] In my studies, I showed clear evidence that 6-CN-flutemetamol labels both non-vascular β-amyloid as well as perivascular β-amyloid (CAA).

6.5.4.2 In vivo ^{18}F-flutemetamol MRI-PET studies

Visual ^{18}F-flutemetamol PET assessment

The overall agreement between the two raters was almost perfect ($\kappa = 0.90$). The one discrepancy was rated positive (based on a single positive right frontal/anterior cingulate region) by one rater but negative by the other.

The raters had different levels of neuroradiology experience; I am a radiology trainee with an interest in neuroradiology while Dr Thompson is a consultant neuroradiologist. We both underwent the Vizamyl™ Electronic Training Programme before assessing PET scans, but neither of us had clinical experience reporting flutemetamol PET images.

This high level of inter-rater agreement is similar to a previous study validating the Vizamyl™ Electronic Training Programme.[309] This study

assessed the reliability of flutemetamol visual assessment by newly trained readers using the electronic training programme. The study participants included healthy volunteers and those with varying degrees of cognitive decline. Inter-rater agreement was very high with most κ scores more than 0.8. Amyloid PET inter-rater agreement may be different in participants with structurally abnormal and asymmetric brains, such as those with ICH. However, my results are similar to the two recent studies assessing florbetapir in ICH, which both showed perfect agreement for visual rating.[303, 304]

Flutemetamol scans are classified visually as positive if a single region is deemed positive. This correlates with the finding that overall visual flutemetamol assessments correlated better with the maximal regional SUVr than global cortical SUVr (Figure 6.16 to Figure 6.18).

The high inter-rater agreement, coupled with perfect discrimination of visual assessment by maximal regional SUVr indicate that visual rating is a reliable and accurate approach for assessing flutemetamol PET scans in ICH. Therefore quantitative analysis may not be necessary for clinical practice.

Diagnostic accuracy of ^{18}F-flutemetamol PET in ICH

The sensitivity of flutemetamol scans rated by visual assessment for probable CAA was 86% (95%CI 42-99). A negative flutemetamol PET scan had a high negative predictive value (91%) and low negative likelihood ratio (0.2), and can effectively rule out advanced CAA.[325] In contrast, a positive scan has moderate specificity 77% (95%CI 46-94), with a positive predictive value of 67% and positive likelihood ratio of 3.7. A positive flutemetamol scan could indicate advanced CAA, Alzheimer's disease or both given the non-specific β-amyloid binding. These estimates are similar to the two recent studies assessing florbetapir for CAA in ICH (pooled sensitivity 90% [95%CI 76-100], specificity 88% [95%CI 74-100]).[326]

Quantitative ^{18}F-flutemetamol uptake in ICH

Previous studies have consistently shown increased global cortical amyloid uptake in CAA-associated ICH versus deep ICH controls.[303, 304, 311] In

my study, global cortical SUVr was higher in the CAA-associated ICH group versus the non-CAA-associated ICH group, although this did not reach statistical significance. This may be because the study is currently underpowered, with only 20 out of the pre-specified 45 sample size included or could reflect misclassifications of the lobar ICH cases by the reference standard as described in 6.5.3.2.

Global cortical SUVr provides a composite measure of flutemetamol uptake, but one can argue that the maximum SUVr is what matters. For example, severe CAA in one region is theoretically sufficient to result in CAA-associated ICH even if globally the burden of CAA is less severe. In line with this, the maximal cortical SUVr in CAA-associated ICH was significantly higher than the non-CAA-associated ICH group.

The significantly reduced occipital/global ratio may be a spurious finding given that a meta-analysis of other amyloid PET studies comparing CAA-associated ICH against deep ICH did not demonstrate a similar association,[327] and this type of regional CAA gradient was not demonstrated in the LINCHPIN brain bank (4.4.6).

6.5.5 Future directions

6.5.5.1 Ex vivo 6-CN-flutemetamol study

My findings need to be replicated in a larger sample. Also, I used participants with absent or severe β-amyloid to assess 6-CN-flutemetamol labelling. Repeating the analyses with tissue from participants with a range of CAA and parenchymal β-amyloid severity will be important to assess whether 6-CN-flutemetamol can label less severe CAA. The extent of 6-CN-flutemetamol labelling in cases of differing CAA severity could be quantified by measuring the fluorescence intensity.

6.5.5.2 In vivo ^{18}F-flutemetamol MRI-PET studies

Although this is an interim analysis of a diagnostic test accuracy study, the findings show the potential diagnostic value of flutemetamol PET in ICH. It is important to continue the study recruitment to reach the pre-specified sample

size. This will allow refinement of the diagnostic test statistics and permit multivariable analysis of SUVr between CAA-associated lobar ICH versus non-CAA-associated ICH groups with adjustment for important confounders, such as age and cognitive status. Finally, there may be sufficient numbers to allow comparison of global cortical SUVr between probable CAA, possible CAA and no CAA categories.

The use of the modified Boston criteria is a pragmatic reference standard in this type of study. However, it will result in misclassification. Assessing amyloid PET uptake against a histopathological reference standard will be a crucial step to determine its true diagnostic accuracy. Currently, 14 of the 20 participants have consented to a research brain autopsy in the event of their death. Comparing the *in vivo* flutemetamol PET to the severity and distribution of CAA at autopsy and *ex vivo* flutemetamol binding will be necessary. There are however challenges to this approach. All the participants recruited to the study were mildly affected by ICH. The time between the index test and autopsy reference standard is therefore likely to be long, and it may be difficult to relate the histopathological changes to the index test. Brain biopsy may, therefore, be a more appropriate source of tissue for future studies of amyloid PET, but it is invasive, infrequently performed and at risk of sampling errors.[164]

Future studies should focus on assessing the value of amyloid PET in clinical practice. A diagnostic accuracy study of the modified Boston MRI criteria, Edinburgh CT-based criteria (Chapter 7) and amyloid PET against a histopathological reference standard is needed to establish the role of these different imaging approaches for diagnosing CAA. It will be important to include ICH participants with a range of pre- and post-ICH disability to evaluate the generalisability of amyloid PET in clinical practice.

One of the limitations of current amyloid PET tracers for clinical practice is the lack of molecular specificity, binding with high affinity to both perivascular and non-vascular β-amyloid.[171, 172] This means a positive scan may relate to Alzheimer's disease, CAA or a combination of the two. Being able to

differentiate CAA from Alzheimer's pathology is vital to improve the specificity of PET in ICH. This cannot be reliably achieved using the distribution of amyloid PET uptake.[327] Research is ongoing to develop a PET tracer which is selective for CAA.[328] Assessment of such a tracer in ICH would be an important further step.

One of the key questions of amyloid PET is whether it can diagnose CAA at an early stage before any haemorrhagic consequences have developed. Longitudinal studies with repeat amyloid PET and MRI scanning will be required to answer this. There are longitudinal amyloid PET studies, such as the Alzheimer's Disease Neuroimaging Initiative (ADNI),[329] which include repeat amyloid PET and MRI scanning in cognitively normal participants. However, the prevalence of severe CAA in non-demented persons aged 70-79 years was only 3% in one community-based neuropathological study,[233] meaning very large sample sizes and long follow up will be required to assess this. An alternative approach would be to study hereditary CAA. In contrast to sporadic CAA, hereditary CAA usually occurs at a young age in selected families carrying specific mutations which are inherited in an autosomal dominant fashion.[18] Amyloid PET and MRI in pre-symptomatic mutation carriers and non-mutation carriers, with longitudinal follow-up for the development of haemorrhagic consequences of CAA, would be feasible in this setting.

Chapter 7 The Edinburgh CT and genetic criteria for lobar ICH associated with CAA: model development and diagnostic test accuracy study

7.1 Introduction

The MRI-based modified Boston criteria are the current non-invasive *in vivo* reference standard for diagnosing CAA and are commonly used in clinical practice.[110, 167] However, patient contraindications to MRI coupled with limited access to MRI scanners in many parts of the world restrict the usefulness of these criteria in ICH. Similarly, the limited availability of PET scanning restricts the value of β-amyloid PET for diagnosing CAA-associated ICH in clinical practice. More easily obtained tests for diagnosing CAA-associated ICH are required.

Non-contrast brain CT is the most widely available neuroimaging modality and usually the first test to diagnose ICH (Figure 3.1). A recent systematic review identified certain imaging features on brain CT, such as subarachnoid haemorrhage and an irregular, lobulated ICH border, are frequently identified in patients with pathologically proven CAA-associated ICH (Section 1.3.4.1).[166] APOE genotyping can be performed using a peripheral blood sample, making it potentially widely available. The presence of an APOE ε4 allele is the strongest genetic association with pathologically proven sporadic CAA, showing a dose-dependent association.[24, 25]

Non-contrast brain CT and APOE genotype may therefore be useful for diagnosing CAA-associated lobar ICH, however their diagnostic accuracy is unknown.

In this chapter I describe the development and internal validation of multivariable diagnostic prediction models for identifying CAA-associated lobar ICH using CT brain features with and without APOE genotype, and the

diagnostic accuracy of simple diagnostic criteria derived from these models. I helped conceive the study design and collect the data. I analysed and interpreted the data and drafted the manuscript.

The Edinburgh CT and genetic diagnostic criteria for lobar intracerebral haemorrhage associated with cerebral amyloid angiopathy: model development and diagnostic test accuracy study

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Summary

Background Identification of lobar spontaneous intracerebral haemorrhage associated with cerebral amyloid angiopathy (CAA) is important because it is associated with a higher risk of recurrent intracerebral haemorrhage than arteriolosclerosis-associated intracerebral haemorrhage. We aimed to develop a prediction model for the identification of CAA-associated lobar intracerebral haemorrhage using CT features and genotype.

Methods We identified adults with first-ever intracerebral haemorrhage diagnosed by CT, who died and underwent research autopsy as part of the Lothian IntraCerebral Haemorrhage, Pathology, Imaging and Neurological Outcome (LINCHPIN) study, a prospective, population-based, inception cohort. We determined *APOE* genotype and radiologists rated CT imaging appearances. Radiologists were not aware of clinical, genetic, and histopathological features. A neuropathologist rated brain tissue for small vessel diseases, including CAA, and was masked to clinical, radiographic, and genetic features. We used CT and *APOE* genotype data in a logistic regression model, which we internally validated using bootstrapping, to predict the risk of CAA-associated lobar intracerebral haemorrhage, derive diagnostic criteria, and estimate diagnostic accuracy.

Findings Among 110 adults (median age 83 years [IQR 76–87], 49 [45%] men) included in the LINCHPIN study between June 1, 2010 and Feb 10, 2016, intracerebral haemorrhage was lobar in 62 (56%) participants, deep in 41 (37%), and infratentorial in seven (6%). Of the 62 participants with lobar intracerebral haemorrhage, 36 (58%) were associated with moderate or severe CAA compared with 26 (42%) that were associated with absent or mild CAA, and were independently associated with subarachnoid haemorrhage (32 [89%] of 36 *vs* 11 [42%] of 26; p=0·014), intracerebral haemorrhage with finger-like projections (14 [39%] of 36 *vs* 0; p=0·043), and *APOE* ε4 possession (18 [50%] of 36 *vs* 2 [8%] of 26; p=0·0020). A prediction model for CAA-associated lobar intracerebral haemorrhage using these three variables had excellent discrimination (c statistic 0·92, 95% CI 0·86–0·98), confirmed by internal validation. For the rule-out criteria, neither subarachnoid haemorrhage nor *APOE* ε4 possession had 100% sensitivity (95% CI 88–100). For the rule-in criteria, subarachnoid haemorrhage and either *APOE* ε4 possession or finger-like projections had 96% specificity (95% CI 78–100).

Interpretation The CT and *APOE* genotype prediction model for CAA-associated lobar intracerebral haemorrhage shows excellent discrimination in this cohort, but requires external validation. The Edinburgh rule-in and rule-out diagnostic criteria might inform prognostic and therapeutic decisions that depend on identification of CAA-associated lobar intracerebral haemorrhage.

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Introduction

About 85% of spontaneous intracerebral haemorrhages have no underlying macrovascular cause and are attributed to small vessel disease, mostly arteriolosclerosis with or without cerebral amyloid angiopathy (CAA).[1,2] CAA affects cortical and leptomeningeal vessels and is only associated with lobar intracerebral haemorrhage,[3,4] whereas arteriolosclerosis can cause intracerebral haemorrhage anywhere in the brain.

Identification of CAA-associated intracerebral haemorrhage is important because it is associated with a higher risk of recurrent intracerebral haemorrhage and post-stroke dementia than arteriolosclerosis-associated intracerebral haemorrhage,[5,6] and might increase the risk of intracerebral haemorrhage in patients taking antithrombotic drugs.[7] Criteria to rule out CAA underlying intracerebral haemorrhage would allow clinicians to be more confident about the use of antithrombotic drugs;[7,7] ruling in CAA underlying intracerebral haemorrhage would provide important prognostic information.

The MRI-based modified Boston criteria have excellent sensitivity and good specificity for CAA.[8] However, MRI

Research in context

Evidence before this study

We did a systematic review of studies on imaging features of lobar or cerebellar intracerebral haemorrhage with pathologically proven cerebral amyloid angiopathy (CAA) published in MEDLINE (from 1946 to Nov 1, 2016) and Embase (from 1974 to Nov 1, 2016) using comprehensive electronic search strategies combining terms "stroke", "cerebrovascular disorders", (brain$ or cerebr$ or intracerebr$) adj5 (h?emorrhag$ or h?ematoma$), "amyloid beta-protein", "cerebral amyloid angiopathy", "vascular amyloidosis", "congo red", "pathology, clinical", "pathology", "histo?patholog$", "post?mortem$" and "autops$" with no language restriction. We identified 22 case series describing imaging features of lobar or cerebellar intracerebral haemorrhage accompanying histopathologically proven CAA. Overall, the study quality was poor, with small sample sizes, unclear definitions of predictor or outcome variables, and infrequent masking of study assessors. The most frequently reported CT features of CAA-associated intracerebral haemorrhage in 21 case series were subarachnoid extension and an irregular intracerebral haemorrhage border. No diagnostic test accuracy studies have been done. Although the modified Boston MRI criteria are widely used for the identification of CAA-associated intracerebral haemorrhage, no CT-based diagnostic criteria exist for patients who cannot tolerate or do not have access to MRI.

Added value of this study

This diagnostic test accuracy study minimised biases, used masking, assessed inter-rater and intra-rater agreement, standardised index test and reference standard, and adhered to recommended approaches for analysis. We were able to develop a highly discriminatory and well calibrated prediction model using subarachnoid haemorrhage and finger-like projections from intracerebral haemorrhage on CT, and *APOE* ε4 possession, which was internally validated. We identified clinically useful probability cutoffs and two sets of diagnostic criteria that can rule in or rule out CAA-associated lobar intracerebral haemorrhage.

Implications of all the available evidence

Both the Boston MRI and the Edinburgh CT-based diagnostic criteria for CAA-associated lobar intracerebral haemorrhage are now available. The Edinburgh sensitive rule-out criteria and specific rule-in criteria based on CT and *APOE* genotype are potentially widely applicable for diagnostic, prognostic, and therapeutic decisions in everyday clinical practice if MRI is contraindicated, intolerable, or unavailable. Future research is required to externally validate these diagnostic criteria and evaluate their clinical use.

can be unsuitable for very unwell patients in the acute setting or for those patients with contraindications, such as non-MRI compatible implanted devices or claustrophobia, and might be unavailable particularly in low-income and middle-income countries where 75% of worldwide deaths due to haemorrhagic stroke occur.[9]

Other tests that can diagnose CAA include CT, which is usually the first test to diagnose intracerebral haemorrhage, and *APOE* genotype. Subarachnoid haemorrhage on CT occurs with 82% (95% CI 69–93) of cases of intracerebral haemorrhage accompanied by histopathologically proven CAA.[10] The presence of an *APOE* ε4 allele is the strongest genetic association with histopathologically confirmed sporadic CAA (odds ratio [OR] 2·67, 95% CI 2·31–3·08), and the association is dose dependent and occurs regardless of dementia comorbidity.[11] However, the diagnostic use of CT features and *APOE* genotype—alone or in combination—is unknown.

We aimed to develop a multivariable prediction model for identifying lobar intracerebral haemorrhage associated with CAA using CT and genetic features, internally validate the model, and assess the diagnostic accuracy of different cutoffs to rule in and rule out CAA-associated intracerebral haemorrhage.

Methods

Study design and participants

We did a community-based inception cohort study of spontaneous intracerebral haemorrhage in the Lothian health board region of Scotland (the Lothian IntraCerebral Haemorrhage, Pathology, Imaging and Neurological Outcome [LINCHPIN] study).[12] We prospectively identified all incident cases of intracerebral haemorrhage with multiple overlapping sources of case ascertainment.[1] We included consecutive adult patients (aged ≥16 years) with first-ever intracerebral haemorrhage confirmed by CT. We excluded patients with recurrent intracerebral haemorrhage; exclusively extra-axial intracranial haemorrhage; and intracerebral haemorrhage secondary to trauma, macrovascular causes, structural causes, or haemorrhagic transformation of an ischaemic stroke.[1] We collected demographics, medical history, and drug use at diagnosis of intracerebral haemorrhage data by interviewing patients or their families or carers at the time of presentation and reviewing primary care and hospital records.[1]

The Scotland A Research Ethics Committee (10/MRE00/23) approved LINCHPIN. We obtained written informed consent from all participants or their immediate next of kin when participants did not have mental capacity.

Index tests

Two neuroradiologists (MAR and PMW) independently evaluated reformatted head CT images with a standardised pro forma derived from previous large-scale stroke studies (appendix).[13] They assessed

See Online for appendix

extra-axial haemorrhage (in the subarachnoid, subdural, or intra-ventricular spaces), finger-like projections (elongated extensions arising from the haematoma, longer than they are wide, regardless of whether they extended to the cortex or not [appendix], variably referred to as lobulated or multinodular appearance in others studies[10,14]), and other radiographic features (appendix). All raters did all assessments masked to clinical, genetic, and pathological information. We used the initial ratings done by MAR for primary analyses.

For *APOE* genotype analysis, we obtained DNA from peripheral blood samples or cerebellar tissue stored in the LINCHPIN brain bank with standard methods described in the appendix. Investigators were masked to clinical, CT, and pathological features during DNA extraction and genotyping (appendix). We classified *APOE* genotype as *APOE* ε2 possession if participants had at least one ε2 allele or *APOE* ε4 possession if they had at least one ε4 allele.

Reference test

One neuropathologist (CS) assessed post mortem brain tissue according to a standard operating procedure (appendix). The maximum interval from death to autopsy was 5 days. The same neuropathologist rated CAA features (appendix) with a consensus rating scale,[15] which rates the presence and severity of parenchymal and meningeal CAA (0–3); the presence of capillary CAA (0 or 1), and vasculopathy (0–2). Two neuropathologists (CS and CH) rated the presence and severity of non-CAA (or other small vessel disease) features in the left cerebral hemisphere using haematoxylin and eosin staining with a modified version of a published scale:[16] none (very mild, occasional arteriolosclerosis without media splitting or luminal narrowing), mild (widespread mild or focal moderate arteriolosclerosis), moderate (widespread moderate or focal severe arteriolosclerosis, with splitting of the media and narrowing of the lumen), or severe (widespread severe arteriolosclerosis, fibrinoid necrosis, lipohyalinosis, evidence of vascular occlusion with or without recanalisation; appendix). We made the CAA ratings similar to the other small vessel disease ratings by restricting them to the parenchymal CAA scores in the left cerebral hemisphere, which we summed and graded for a CAA burden category (0=none, 1–4=mild, 5–8=moderate, and 9–12=severe). Macroscopic neuropathological assessment could not be masked to intracerebral haemorrhage location, but investigators were masked to other CT features, clinical information, and genotype. For microscopic histopathological assessment, investigators were masked to intracerebral haemorrhage location when possible (ie, unless the intracerebral haemorrhage was included in one of the prespecified sampled regions), as well as other CT features, clinical information, and genotype.

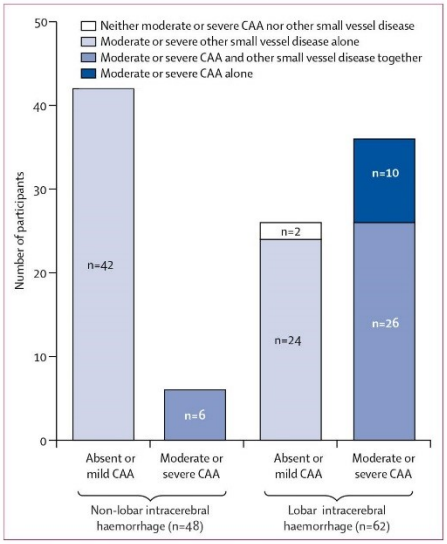

Figure 1: **Pathological severity of CAA and other small vessel disease according to intracerebral haemorrhage location**
CAA=cerebral amyloid angiopathy.

For more on **VassarStats Clinical Calculator 1** see http://vassarstats.net/clin1.html

Statistical analysis

We were unable to calculate the sample size required for a diagnostic test accuracy study because a systematic review[10] identified only two retrospective cross-sectional studies that compared CT features of intracerebral haemorrhage associated with histopathologically proven CAA with intracerebral haemorrhage without CAA, but these two studies were at high risk of selection and information biases. Therefore, after completing recruitment to this prospective population-based study,[1] we used the largest sample size possible (n=110) from its nested brain bank and we restricted models to three predictors (nine outcomes per variable) to reduce overfitting.[17] We did a post-hoc power calculation using a two-sided Z test for a logistic regression model with a 5% level of significance, with subarachnoid haemorrhage as the main predictor (OR 10·9) and adjusting for the effect of other covariates (Nagelkerke R^2 0·06); this calculation showed that our maximum achievable sample size of 62 participants with lobar intracerebral haemorrhage would result in 70·6% power.

No data were missing from this study. We did statistical analyses using the *R* statistic package (version 3.3.2), with the exception of post-hoc power calculation and diagnostic accuracy statistics (sensitivity, specificity, likelihood ratios, and predictive values and their 95% CIs) which we obtained using G*Power 3.1.9.2 and VassarStats Clinical Calculator 1, respectively.

Prediction modelling and performance

The multivariable prediction model aimed to use CT features and *APOE* genotype to predict lobar intracerebral haemorrhage that was associated with CAA, defined as a CAA burden category of moderate or severe, with or without other small vessel disease.

We assessed intra-rater and inter-rater agreement of CT features using unweighted Cohen's κ coefficient for categorical data, linear-weighted Cohen's κ coefficient for ordinal data, and intra-class correlation coefficient for continuous data. We excluded radiographic features with κ less than 0·5 from further analyses. We compared the distributions of clinical, genetic, and CT characteristics in cases of lobar intracerebral haemorrhage with or without moderate or severe CAA using χ^2 test (or Fisher's exact test, where appropriate) for categorical variables and the Mann-Whitney *U* test for non-normally distributed continuous variables. For the full model we prespecified subarachnoid haemorrhage and *APOE* ε4 possession on the basis of systematic review data.[10,11] We included finger-like projections from the intracerebral haemorrhage on the basis of the strong univariate association that we found with CAA-associated lobar intracerebral haemorrhage. We used Firth's penalised likelihood logistic regression to assess the association of predictors with CAA-associated lobar intracerebral haemorrhage and calculate OR with 95% CIs, because finger-like projections showed complete separation between the two outcome groups.[18] We assessed overall performance using Nagelkerke's R^2, the Brier score, and the Akaike information criterion.[19] We evaluated model discrimination with the concordance (c) statistic and discrimination slope, and displayed the discrimination graphically using a receiver operating characteristic plot. We assessed model calibration with the Hosmer–Lemeshow goodness-of-fit test and calibration plots. We used bootstrapping to evaluate the internal validity of the model performance measures.[20] We used 2000 random bootstrap samples with replacement from the full sample of participants with lobar intracerebral haemorrhage, constructed models on these bootstrap samples, and derived optimism-adjusted performance measures to provide a realistic estimate of future performance.[19]

Development of diagnostic criteria

We defined three risk categories for CAA-associated lobar intracerebral haemorrhage according to the probability of CAA-associated intracerebral haemorrhage predicted by the model: low (≤7%), medium (44–64%), and high (≥95%). We used decision curve analysis, which assesses the use of different risk category cutoffs across the full range of threshold probabilities and false positive and false negative weighting, to confirm the optimum risk category cutoffs for ruling CAA-associated intracerebral haemorrhage either in or out.[21] We evaluated the sensitivity, specificity, positive and negative likelihood ratios, and predictive values and their 95% CIs for the

	Absent or mild CAA (n=26)	Moderate or severe CAA (n=36)	Odds ratio (95% CI)	p value
Age (years)	84 (78–88)	82 (79–85)	NC	0·41
Sex				
Men	12 (46%)	11 (31%)	0·51 (0·18–1·46)	0·27
Women	14 (54%)	25 (69%)	1·95 (0·68–5·55)	0·21
Hypertension	19 (73%)	23 (64%)	0·65 (0·22–1·96)	0·45
Antiplatelet use at intracerebral haemorrhage	15 (58%)	18 (50%)	0·73 (0·27–2·03)	0·55
Anticoagulant use at intracerebral haemorrhage	4 (15%)	5 (14%)	0·89 (0·21–3·68)	1·00
Dementia	2 (8%)	8 (22%)	3·43 (0·66–17·72)	0·17
APOE ε2 possession	3 (12%)	11 (31%)	3·37(0·83–13·63)	0·077
APOE ε4 possession	2 (8%)	18 (50%)	12·00 (2·46–58·47)	0·0004
Multiple intracerebral haemorrhage	6 (23%)	3 (8%)	0·30 (0·07–1·35)	0·15
Left side	14 (54%)	18 (50%)	0·86 (0·31–2·35)	0·76
Intracerebral haemorrhage location				
Frontal	10 (38%)	19 (53%)	1·79(0·64–4·99)	0·27
Parietal	6 (23%)	8 (22%)	0·95(0·29–3·17)	0·94
Temporal	5 (19%)	5 (14%)	0·68(0·17–2·63)	0·57
Occipital	5 (19%)	4 (11%)	0·53(0·13–2·18)	0·38
Intracerebral haemorrhage volume (mL)	59 (23–126)	66 (22–117)	NC	0·72
Strictly lobar intracerebral haemorrhage	22 (85%)	36 (100%)	NA	0·027
Intraventricular extension	14 (54%)	17 (47%)	0·77 (0·28–2·11)	0·61
Any subarachnoid haemorrhage	11 (42%)	32 (89%)	10·91 (2·98–39·96)	<0·0001
Subdural extension	5 (19%)	7 (19%)	1·01 (0·28–3·64)	0·98
Midline shift	18 (69%)	21 (58%)	0·62 (0·21–1·80)	0·38
Finger-like projections	0	14 (39%)	34·16 (1·93–605·23)	0·0003
Cortical involvement	21 (81%)	35 (97%)	8·33 (0·91–76·28)	0·074
Dilute or seeping	9 (35%)	15 (42%)	1·35 (0·47–3·84)	0·59
Old vascular lesion	8 (31%)	15 (42%)	1·61 (0·55–4·66)	0·38
Anterior WML	..	..	..	0·26
0	2 (8%)	8 (22%)	..	..
1	16 (62%)	21 (58%)	..	..
2	8 (31%)	7 (19%)	..	..
Posterior WML	..	..	..	0·65
0	7 (27%)	6 (17%)	..	..
1	3 (12%)	6 (17%)	..	..
2	16 (62%)	24 (67%)	..	..
Central atrophy	..	..	..	0·26
0	9 (35%)	10 (28%)	..	..
1	17 (65%)	22 (61%)	..	..
2	0	4 (11%)	..	..
Cortical atrophy	..	..	..	0·37
0	4 (15%)	11 (31%)	..	..
1	15 (58%)	18 (50%)	..	..
2	7 (27%)	7 (19%)	..	..

Data are n (%) or median (IQR). CAA=cerebral amyloid angiopathy. NC=not calculable because the data are continuous. NA=not available as one or more cells contained a zero. WML=white matter lesion.

Table 1: **Characteristics of lobar intracerebral haemorrhage associated with severity of CAA**

diagnostic criteria that distinguished low versus medium or high risk categories, and high versus medium or low risk categories.

For the **data used in this study** see http://dx.doi.org/10.7488/ds/2230

	β coefficient (SE)	Odds ratio (95% CI)	p value
Intercept	−2·55 (0·89)	..	0·0040
APOE ε4 carrier	3·11 (1·01)	22 (4–862)	0·0020
Subarachnoid haemorrhage	2·31 (0·94)	10 (2–299)	0·014
Finger-like projections	3·20 (1·58)	27 (3–not reached)	0·043

Table 2: **Multivariable Firth's logistic regression prediction model for lobar intracerebral haemorrhage associated with moderate or severe cerebral amyloid angiopathy**

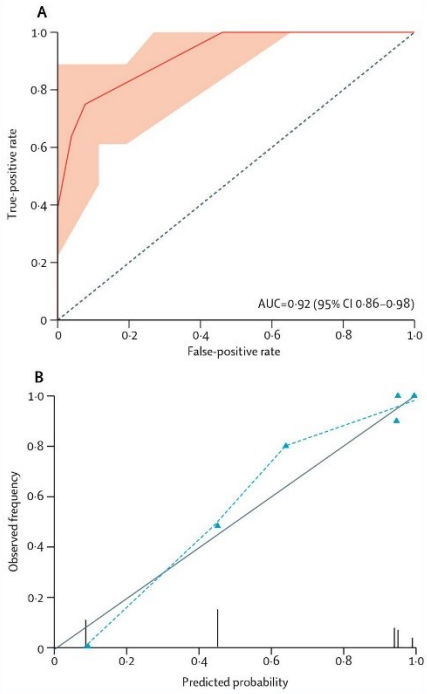

Figure 2: **Discrimination and calibration measures of prediction model performance**
(A) Receiver operating characteristic curve for predicted probability of moderate or severe CAA. The AUC is equivalent to the c statistic. The shaded area represents the 95% CI of the AUC based on 2000 bootstrap replicates. The dotted line indicates a non-informative AUC of 0·50 for comparison. (B) Calibration plot of predicted probability versus observed frequency of moderate or severe CAA. Grey line indicates perfect calibration, the model's calibration is shown by the dotted line. Triangles represent the six different moderate or severe CAA risk groups produced by the prediction model. Vertical lines represent the frequency and distribution of model predicted probabilities. CAA=cerebral amyloid angiopathy. AUC=area under the curve.

Data sharing

Clinical, radiographic, genetic, and pathological data used in this study are available online, along with the code for logistic regression and internal validation.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between June 1, 2010, and Feb 10, 2016, 110 participants underwent research autopsy after non-contrast head CT that initially diagnosed first-ever intracerebral haemorrhage, after unavoidable exclusions (appendix).[12] The median age was 83 years (IQR 76–87) and 49 (45%) were men (appendix). DNA for *APOE* genotyping was available for all 110 participants (peripheral blood sample for 28 participants and post-mortem cerebellar tissue for 82 participants). The median time from intracerebral haemorrhage to CT was 5 h (IQR 3–18) and the median time from CT to autopsy was 11 days (5–80). For most radiographic features, intra-rater agreement was substantial to almost perfect and inter-rater agreement was moderate to almost perfect (appendix).

62 (56%) participants had lobar intracerebral haemorrhage, 41 (37%) had deep intracerebral haemorrhage, and seven (6%) had infratentorial intracerebral haemorrhage (appendix). All 48 participants with non-lobar intracerebral haemorrhage had moderate or severe other small vessel disease: most (n=42 [88%]) had other small vessel disease with absent or mild CAA and six (13%) also had moderate or severe CAA, consistent with the population prevalence of CAA in octogenarians (figure 1, appendix).[2] Therefore, we focused our further analyses on the prediction of moderate or severe CAA in participants with lobar intracerebral haemorrhage.

Of 62 participants with lobar intracerebral haemorrhage, 26 (42%) had moderate or severe other small vessel disease as well as moderate or severe CAA, 24 (39%) had moderate or severe other small vessel disease alone, ten (16%) had moderate or severe CAA alone, and two (3%) had no clear underlying cause of intracerebral haemorrhage (one participant had mild CAA and mild other small vessel disease, and the second participant had mild other small vessel disease and absent CAA, but neither had a macrovascular abnormality, coagulopathy, or tumour; figure 1). In univariable analyses, participants with lobar intracerebral haemorrhage and moderate or severe CAA were significantly more likely to be *APOE* ε4 carriers, and to have a strictly lobar intracerebral haemorrhage, subarachnoid haemorrhage, and finger-like projections from the intracerebral haemorrhage than participants with lobar intracerebral haemorrhage and absent or mild CAA (table 1).

The multivariable prediction model for CAA-associated lobar intracerebral haemorrhage included three predictors: *APOE* ε4 possession, subarachnoid haemorrhage, and finger-like projections (table 2). The model calculates the predicted probability of moderate or severe CAA as follows (the predictor values are 1 when present and 0 when absent):

$$\text{Predicted probability} = \frac{1}{1+\exp^{-\text{risk score}}}$$

Risk score = −2·55 + 3·11 × (*APOE* ε4 positive) + 2·31 × (subarachnoid haemorrhage) + 3·20 × (finger-like projections)

All three predictors were independently associated with moderate or severe CAA. The variance inflation factor values (*APOE* ε4 carrier 1·33, subarachnoid haemorrhage 1·34, and finger-like projections 1·03) confirmed no evidence of multicollinearity between predictors. We did a sensitivity analysis excluding participants taking oral anticoagulants (n=9), given the high frequency of finger-like projections in such cases,[22] which showed similar significance, direction, and magnitude of the independent associations (appendix).

The model showed excellent discrimination (c statistic 0·92, 95% CI 0·86–0·98) with no evidence of poor calibration (Hosmer–Lemeshow goodness of fit test p=0·685; figure 2, appendix). Internal validation identified small differences in the overall performance measures (eg, the Brier score increased from 0·11 to 0·12 and the c statistic decreased from 0·92 to 0·91), with no evidence of poor calibration (appendix).

We used the multivariable model to select two cutoff points to stratify the probability of moderate or severe CAA into low, medium, or high risk (appendix). When no predictors were present (low risk), the predicted probability of moderate or severe CAA was 7%. Subarachnoid haemorrhage or *APOE* ε4 possession in isolation (medium risk) predicted 44–64% probability of moderate or severe CAA. The presence of subarachnoid haemorrhage and at least one other predictor (high risk) predicted a probability of moderate or severe CAA of 95% or more.

Guided by how the predictors stratified the probability of moderate or severe CAA associated with lobar intracerebral haemorrhage, we identified two sets of diagnostic criteria including the three predictors (appendix, figure 3). Subarachnoid haemorrhage or *APOE* ε4 possession separated low from medium or high probability groups (appendix), and had a sensitivity of 100% (95% CI 88–100; appendix) meaning that the absence of these two predictors with lobar intracerebral haemorrhage ruled out moderate or severe CAA. Subarachnoid haemorrhage and *APOE* ε4 possession or finger-like projections, separated high from low or medium probability groups (appendix), and had a specificity of 96% (95% CI 78–100; appendix) meaning that the presence of these predictors with lobar intracerebral haemorrhage ruled in moderate or severe CAA. These optimum risk category cutoffs for ruling CAA-associated intracerebral haemorrhage either in or out were confirmed by decision curve analysis (appendix).

We repeated the prediction modelling and derived diagnostic criteria using CT ratings from a second independent investigator (PMW). The prediction model shows similar direction and magnitude of the predictors with moderate or severe CAA, while the sensitivity of the rule-out criteria remained 100% and specificity of the rule-in criteria increased to 100% (appendix).

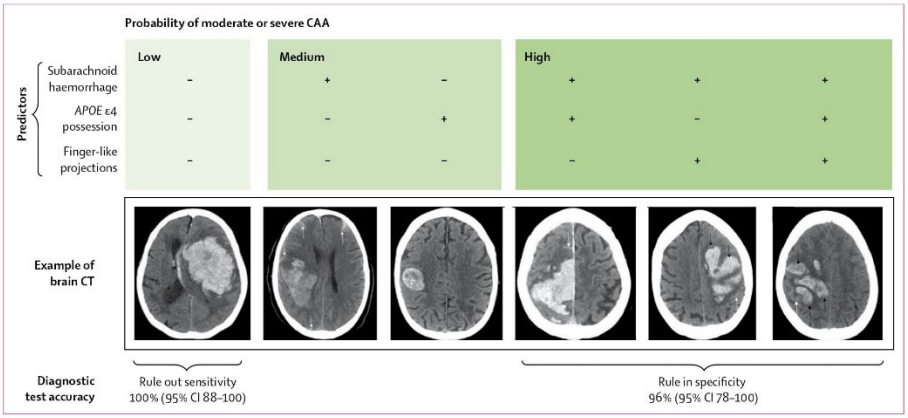

Figure 3: **Categorisation of probability of lobar intracerebral haemorrhage associated with moderate or severe cerebral amyloid angiopathy according to the three predictor variables, with example CT images**
CAA=cerebral amyloid angiopathy. Adapted from Salman and Rodrigues (Creative Commons 4.0).[23]

We did a comparison of our CT and genetic criteria with the modified Boston criteria[8] in the seven participants with lobar intracerebral haemorrhage who subsequently had MRI done within 6 months of the intracerebral haemorrhage, and found that all cases at high or intermediate probability of having moderate or severe CAA by our Edinburgh criteria were classified as probable CAA by the Boston criteria (appendix).

Since *APOE* genotyping might not be available worldwide, we assessed the diagnostic test accuracy of a simplified model on the basis of CT features alone (appendix). Subarachnoid haemorrhage alone had a sensitivity of 89% (95% CI 73–96) and the combination of subarachnoid haemorrhage and finger-like projections had 100% (95% CI 84–100) specificity (appendix). However, the inclusion of *APOE* ε4 improved the model (full model χ^2=59·0, Akaike information criterion=49·9 *vs* simplified CT-based model χ^2=41·9, Akaike information criterion=65·0, p<0·0001).

Discussion

In our study, we used a systematically acquired brain tissue bank nested within a prospective, population-based cohort study to develop and internally validate a simple, three-variable model using two CT features (subarachnoid haemorrhage and finger-like projections from intracerebral haemorrhage) and *APOE* genotype to predict moderate or severe CAA associated with lobar intracerebral haemorrhage. The model had excellent discrimination and calibration. The diagnostic criteria might inform estimates of prognosis and decisions about antithrombotic drugs after intracerebral haemorrhage.

We identified two clinically useful diagnostic cutoffs that have implications for clinical practice. Neither subarachnoid haemorrhage nor *APOE* ε4 possession was 100% sensitive for moderate or severe CAA with a negative likelihood ratio of 0; a negative likelihood of less than 0·1 means a negative test is good at ruling out a diagnosis.[24] Therefore, the absence of these features can rule out CAA-associated lobar intracerebral haemorrhage, which might identify people with a lower risk of recurrent intracerebral haemorrhage,[5,25] dementia,[6] and susceptibility to the effects of antithrombotic drugs.[7] The presence of subarachnoid haemorrhage and *APOE* ε4 possession or finger-like projections was 96% specific with a positive likelihood ratio 16·6. A positive likelihood of more than 10 means a positive test is good at ruling in a diagnosis,[24] so the presence of these features can effectively rule in CAA-associated lobar intracerebral haemorrhage to identify patients for studies of CAA treatment.

Our diagnostic criteria are based on features identified on non-contrast CT, a widely available, relatively inexpensive diagnostic test with few contraindications, which is suitable in acutely unwell patients. The intra-rater and inter-rater agreement for subarachnoid haemorrhage and finger-like projections were moderate to almost perfect. Although *APOE* genotyping using peripheral blood samples is not a universally available test, the inclusion of *APOE* ε4 possession significantly improves the prediction model. Given the drive towards stratified medicine, these techniques are becoming increasingly cost-effective.[26] Our criteria could be used in other patient groups to help guide the use of more restricted clinical resources by identifying patients who might benefit from advanced imaging, such as MRI or PET to assess for further features of CAA and other small vessel disease biomarkers.

This study minimised selection bias by using prospective case ascertainment in one community and inviting all potentially eligible people to consent to the nested brain bank. We minimised information bias by standardising imaging format; defining and systematically assessing radiographic features; standardising brain tissue acquisition; extensively sampling brain tissue, rather than using cortical biopsy; systematically assessing pathological features; using validated rating scales;[15,16,27] and masking assessors. No data were missing and inter-rater agreement was moderate to substantial for the key predictors. We chose logistic regression rather than machine-learning approaches, such as neural networks, for our prediction model, because of its simplicity, familiarity, and transparency. We reduced overfitting by restricting the multivariable model to three predictors, prespecified two of these predictors, and avoided stepwise methods of predictor selection due to the probable instability of selection related to the sample size. We did internal validation with bootstrapping to further reduce optimism in study performance measures.

This study has some limitations. Sample size was modest and the post-hoc power calculation shows that the study has 71% power and therefore is at risk of a type II error. Our sample size left us unable to develop criteria to distinguish lobar intracerebral haemorrhage associated with CAA alone, other small vessel disease alone, and mixed CAA and other small vessel disease, which could be clinically important. However, this study has the largest sample we could achieve over 6 years in a population of about 850 000 people, with roughly 50% consenting soon after an acute brain injury,[12] which is comparable with other brain banks.[28]

Although we tried to limit selection bias, participants who underwent autopsy were older, had larger intracerebral haemorrhage, more frequent intraventricular extension, and more severe posterior white matter lucencies, and generally died soon after their intracerebral haemorrhage compared with participants who did not (appendix); these differences were inevitable in standard clinical practice, and indicate to whom our results are generalisable. Whilst the frequency of model predictors (*APOE* ε4 possession, subarachnoid haemorrhage and finger-like projections) and the distribution of risk categories did not vary between those participants who underwent autopsy and those participants who did not undergo autopsy (appendix), the applicability of our

criteria to other intracerebral haemorrhage groups—such as younger patients with smaller intracerebral haemorrhage—is unclear.

We only identified finger-like projections in cases of lobar intracerebral haemorrhage with moderate or severe CAA, making them very specific for CAA-associated haemorrhage. However, finger-like projections are difficult to define, subjective, and potentially prone to observer variability. We used both written and pictorial definitions to improve reliability and showed substantial intra-rater and moderate inter-rater agreement. Furthermore, we repeated the modelling using ratings from a second independent investigator, which resulted in rule-out criteria with 100% sensitivity and rule-in criteria with 100% specificity for CAA-associated lobar intracerebral haemorrhage. Previous studies[10] have reported that irregular and lobulated intracerebral haemorrhage borders are more frequently associated with CAA-associated lobar intracerebral haemorrhage. However, these features were not defined in the studies, making them difficult to relate to our findings.

Finally, CT features of intracerebral haemorrhage, such as subarachnoid haemorrhage and finger-like projections, will evolve with time. The timecourse for this evolution in intracerebral haemorrhage is unknown and how it might affect the diagnostic accuracy of these criteria is unclear. We would expect a reduction in sensitivity with time, however our rule-out criteria remain 100% sensitive despite including participants who had a CT scan up to 7 days after symptom onset.

We are planning an external validation study to assess the performance of the prediction model and diagnostic criteria in other settings, countries, ethnic groups, and patient populations, such as those patients with smaller intracerebral haemorrhage, and determine the reproducibility of predictor assessment by other investigators (Greenberg SM, Massachusetts General Hospital, Boston, MA, USA, personal communication). Further large, representative samples with a systematic reference standard based on autopsy could attempt to distinguish lobar intracerebral haemorrhage associated with CAA alone, other small vessel disease alone, and mixed CAA and other small vessel disease. Comparison of these CT and genetic criteria and the widely used modified Boston criteria[8] against a pathological reference standard would be noteworthy. Studies of the clinical and economic impact of these criteria on prognosis and therapeutic decisions might quantify, and hopefully confirm, their effect on the outcome of this devastating disease.

Contributors

MAR did the literature search, conceived the study design, collected, analysed, and interpreted the data, made the figures, drafted the manuscript, and had final approval of the manuscript. NS did the literature search, conceived the study design, collected data, and critically revised the manuscript for important intellectual content. CL, CH, and PMW collected data and critically revised the manuscript for important intellectual content. MOM, JARN, CLMS, and CC designed the study and critically revised the manuscript for important intellectual content. JMW designed the study, collected and interpreted data, and critically revised the manuscript for important intellectual content. CS collected and interpreted data, and critically revised the manuscript for important intellectual content. RA-SS conceived the study design, interpreted the data, and critically revised the manuscript for important intellectual content. All authors gave final approval of the manuscript.

Declaration of interests

CH reports grants from Alzheimer's Society, during the conduct of the study; and grants from Alzheimer's Research UK, outside the submitted work. PMW reports grants from Medical Research Council, during the conduct of the study; and sits on the Stryker Neurovascular Clinical Advisory Board whose remit includes intracerebral haemorrhage. CC has participated on national boards for Bayer, Medtronics, and Daichii-Sankyo; has been a clinical investigator in trials supported by AstraZeneca, Daichii-Sankyo, and Boehringer Ingelheim; and was national coordinator and investigator for the study A9951024 supported by Pfizer, outside the submitted work. JMW reports grants from European Union Horizon 2020, Fondation Leducq, Medical Research Council, Engineering and Physical Sciences Research Council, British Heart Foundation, The Stroke Association, The Alzheimer's Society, The Chief Scientist Office, and the Wellcome Trust, outside the submitted work. All other authors declare no competing interests.

Acknowledgments

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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Rodrigues MA, Samarasekera N, Lerpiniere C, et al. The Edinburgh CT and genetic diagnostic criteria for lobar intracerebral haemorrhage associated with cerebral amyloid angiopathy: model development and diagnostic test accuracy study. *Lancet Neurol* 2018; published online Jan 10. http://dx.doi.org/10.1016/S1474-4422(18)30006-1.

Index test methods

CT head – reformatting

One trainee neuroradiologist (MAR) reformatted the first diagnostic non-contrast head CT scan performed after ICH onset into standard axial (parallel to a line linking the floor of the sella turcica to the fastigium of the fourth ventricle), coronal (parallel to the posterior surface of the brain stem) and sagittal (parallel to the interhemispheric fissure) planes, with standard slice thickness (5mm), spacing (3mm), and windowing (centre 35, width 80).

CT rating proforma

LINCHPIN CT RATING FORM

P1

LINCHPIN ID: _____ SCAN DATE: _____

READ DATE: _____ RATER INITIALS: _____

IMAGE SERIES: *Significant series (unenhanced 5mm Axial, coronal & sagittal reformats)*

Grade overall image quality: Poor Adequate Good

1.	Is there any sign of acute ischaemic change?	Y	N	IF NO, GO TO Q.2
a.	On which side of the brain is the ischaemia?	R	L	BOTH
2.	Is there any acute parenchymal haemorrhage?	Y	N	IF NO, GO TO Q.8
a.	Is the main haemorrhage most likely Haemorrhagic Transformation of an Infarct or Primary Intracerebral Haemorrhage?	HTI	PICH	IF HTI, GO TO Q.8
3.	Are there multiple sites of acute parenchymal haemorrhage?	Y	N	

a. Characterize each separate acute parenchymal haemorrhage starting with the largest:

	1	2	3	4	5
Left/Right/ Central					
Anatomic Location code(s) (site(s) are which the ICH is thought to be centred)					
Deep (D), Lobar (L), Mixed lobar & deep (M), Infratentorial (IT) Uncertain (U)					

Anatomic Location Codes for haemorrhages

LOBAR		**DEEP**	
F	Frontal lobe (not basal ganglia)	BG	Basal ganglia (not thalamus)
TE	Temporal lobe	TH	Thalamus
PA	Parietal lobe	IC	Internal capsule
O	Occipital lobe	EC	External capsule
INFRATENTORIAL		WM	Deep & periventricular WM and corpus callosum
CE	Cerebellum		
BS	Brainstem (Midbrain, pons, medulla)		

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SCAN ID: _____ LINCHPIN CT P2

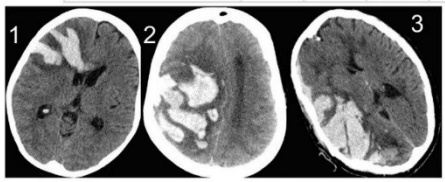
b.	Is there any intraventricular haemorrhage?	Y	N	
c.	Is there any subarachnoid extension?	Y	N	IF YES, GO TO 1D. IF NO, GO TO 1E
d.	If there is subarachnoid extension, is this adjacent to the ICH? Record If distant or both	Y	N	
e.	Is there any subdural extension?	Y	N	
f.	Is there any midline shift or herniation?	Y	N	

4. Is there a blood/fluid level within any parenchymal haemorrhage? (NOT including an intraventricular fluid level; start with the largest first)

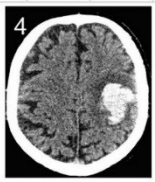
	1	2	3	4	5
Blood or fluid level (Y/N)					

5. Describe the border and shape of each separate acute parenchymal haemorrhage starting with the largest first (see examples below)

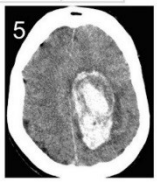
	1	2	3	4	5
a. Irregular border? (Y/N)					
b. Finger-like protrusions? (Y/N)					
c. Round/oval? (Y/N)					
d. Reaches cortex? (Y/N)					



Images 1-3
Irregular border with **finger-like protrusions** to **cortex**



Images 4
Irregular border, reaches **cortex without** finger-like protrusions



Images 5
Regular border **without** finger-like protrusions & **does not** reach cortex

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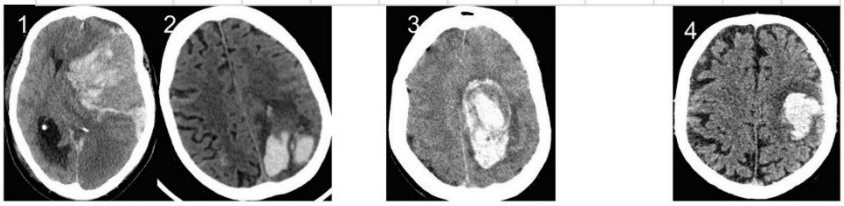
SCAN ID: _____ LINCHPIN CT P3

6.	Describe the density of each acute parenchymal haemorrhage starting with the largest first					
	1	2	3	4	5	
a. Variable density? (Y/N)	☐	☐	☐	☐	☐	
b. Dilute/seeping appearance? (Y/N)	☐	☐	☐	☐	☐	



Variable density & dilute/seeping (1-2)
Blood in any single ICH has variable **density** & appears 'dilute'/seeping into adjacent brain tissue

Variable density (3)
Blood in any single ICH has variable **density** but not 'dilute'

Uniform density (4)
Blood in any single ICH has uniform **homogeneous density**

7.	Characterize the parenchymal haemorrhage(s) listed in 3 further. (Do not include extra-axial haemorrhage in the measurements)			
a.	What is the size of haemorrhage no.1 (mm)? A is largest diameter in the axial plane, B is longest axis perpendicular to A in the axial plane, C is maximum diameter in craniocaudal plane	A ☐	B ☐	C ☐
b.	What is the size of haemorrhage no.2 (mm)?	A ☐	B ☐	C ☐
c.	What is the size of haemorrhage no.3 (mm)?	A ☐	B ☐	C ☐
d.	What is the size of haemorrhage no.4 (mm)?	A ☐	B ☐	C ☐
e.	What is the size of haemorrhage no.5 (mm)?	A ☐	B ☐	C ☐

8.	Are there any **old** vascular lesions?	Y ☐	N ☐	IF NO, GO TO Q.9
a.	Classify the old vascular lesions			

	A	B	C	D	E	F	G
Code Y/N	☐	☐	☐	☐	☐	☐	☐

A = old cortical infarct(s) B = old striatocapsular infarct(s) C = old borderzone infarct(s)

D = old lacunes E = old brainstem/cerebellar infarct(s) F = probable old **deep** haemorrhage

G = probable old **lobar** haemorrhage

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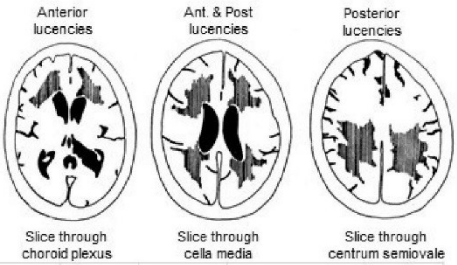
SCAN ID: _____ LINCHPIN CT P4

9.	Classify any PERIVENTRICULAR LUCENCIES			[match "template" below]

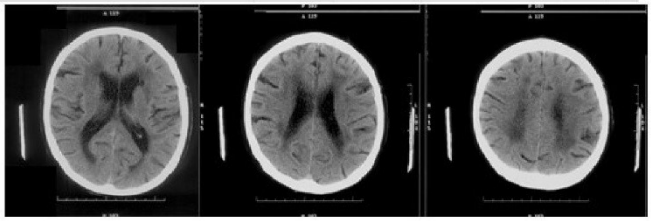
diagram from van Swieten et al. JNNP 1990;53:1080-1083



Rating white matter lucency:
0= no lucency
1= lucency restricted to region adjoining ventricles
2= lucency covering entire region from lateral ventricle to cortex

a.	Anterior white matter		0,1,2	Overall ☐
b.	Posterior white matter		0,1,2	Overall ☐

Exemplar:
AWM = 2 PWM = 1
Source:
http://www.sbirc.ed.ac.uk/documents/ctandmr%20reading%20form.pdf



10	Rate any ATROPHY 0 = None. 1 = Moderate 2= Severe			[match "template" below]
a.	Central (deep)			Overall ☐

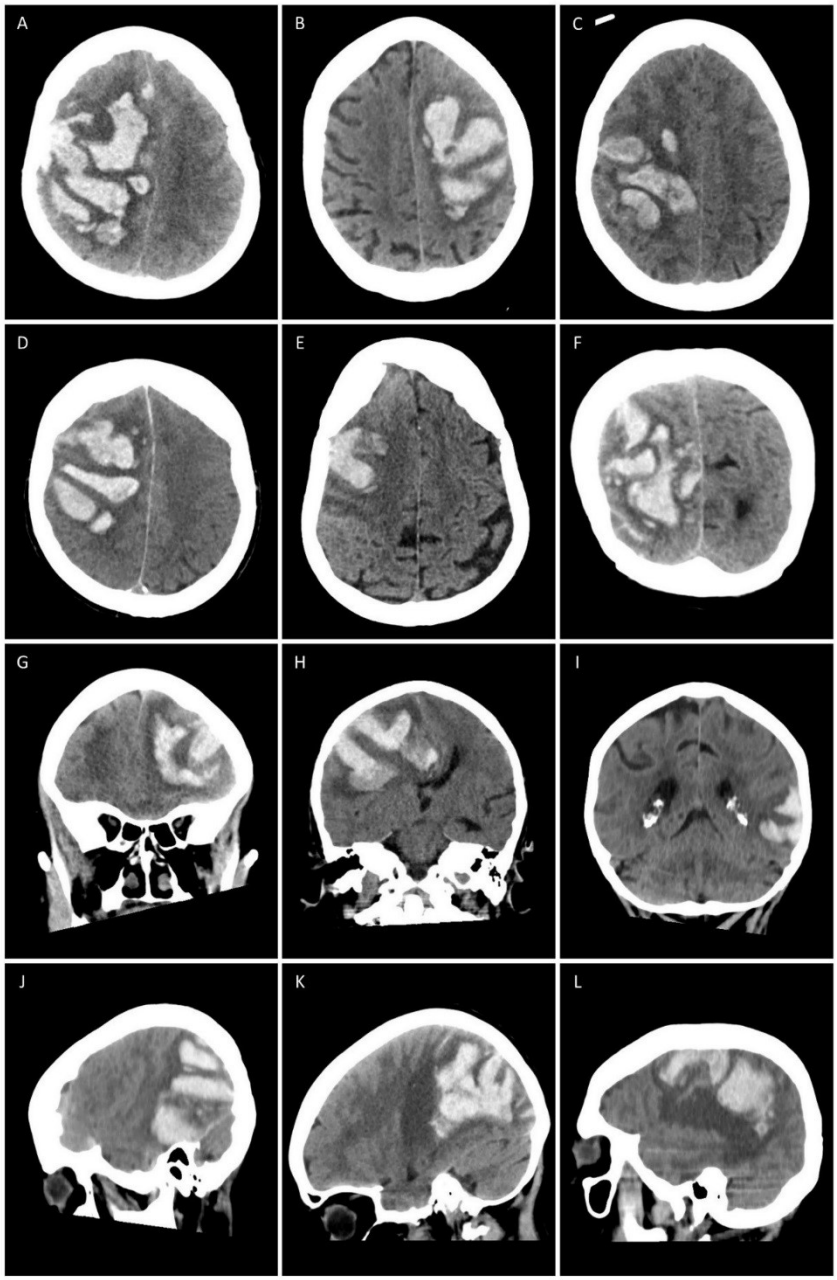
Source:
http://www.sbirc.ed.ac.uk/documents/ctandmr%20reading%20form.pdf

b.	Cortical			Overall ☐

11	Comments

LINCHPIN CT Rating form v.3 07/04/16 – Derived with permission from IST3/LSR

12 separate case illustrations of finger-like projections



A-E: axial plane; F-I: coronal plane; J-L: sagittal plane

CT head – rating of other radiographic features

ICH volume was assessed using the *ABC/2* method where *A* is the largest diameter of ICH on in the axial plane (cm), *B* is the largest diameter at 90° to *A* on the same slice (cm), and *C* is the maximal cranio-caudal diameter (cm).[1]

ICH shape (e.g. irregular contour, finger-like projections) and appearance (e.g. fluid level and dilute/"seeping" density) was assessed.

We recorded the presence or absence of old infarcts, severity of anterior and posterior white matter lucencies using the van Swieten scale,[2] deep (enlargement of the ventricles) and superficial (enlargement of the sulci) cerebral atrophy using a template based three-point scale (absent/mild, moderate and severe).[3]

We used representative pictures to define specific imaging features – such as irregular contour, finger-like projection, dilute/"seeping" density and atrophy grades – which we defined as present or absent.

One trainee neuroradiologist (MAR) recorded ICH location using the Cerebral Haemorrhage Anatomical RaTing Scale (CHARTS),[4] and re-assessed the scans after a period of three months to evaluate intra-observer agreement.

DNA extraction and APOE genotyping

DNA was extracted from whole blood using a Nucleon Kit (GenProbe) with the BACC3 protocol. DNA samples were re-suspended in 1 ml TE buffer pH 7·5 (10mM Tris-Cl pH 7·5, 1mM EDTA pH 8·0). The yield of the DNA was measured using picogreen and normalised to 10ng/µl before genotyping. DNA was extracted from fresh-frozen brain tissue by homogenising using buffer ATL with proteinase K and incubating at 56°C on a thermomixer at 1000 rpm then isolated using Qiagen DNeasy blood and tissue kit. DNA samples were re-suspended in 200µl of Qiagen elution buffer and normalised to 10ng/µl before genotyping. DNA was extracted from formalin fixed paraffin embedded tissue brain tissue using the Covaris E220 Focused Ultra Sonicator and the truXTRAC FFPE DNA kit, following the genomic DNA extraction protocol. 20µm tissue scrolls were deparaffinised by sonication for 2 x 5 minute periods before overnight incubation on a thermomixer at 56°C with proteinase K. Crosslinking was reversed by incubation at 80°C for 1 hour before purification in spin columns and elution in 50µL of Covaris Buffer BE (5mM Tris HCl pH 8·5).

Genotypes for two APOE single-nucleotide polymorphisms (rs429358 and rs7412) were determined using TaqMan single-nucleotide polymorphism genotyping assays (Applied Biosystems, Foster City, CA) on a ThermoFisher QuantStudio 12K Flex Real Time PCR System instrument with QuantStudio 12K Flex Software or Taqman Genotyper Software v1·3.

Reference test methods

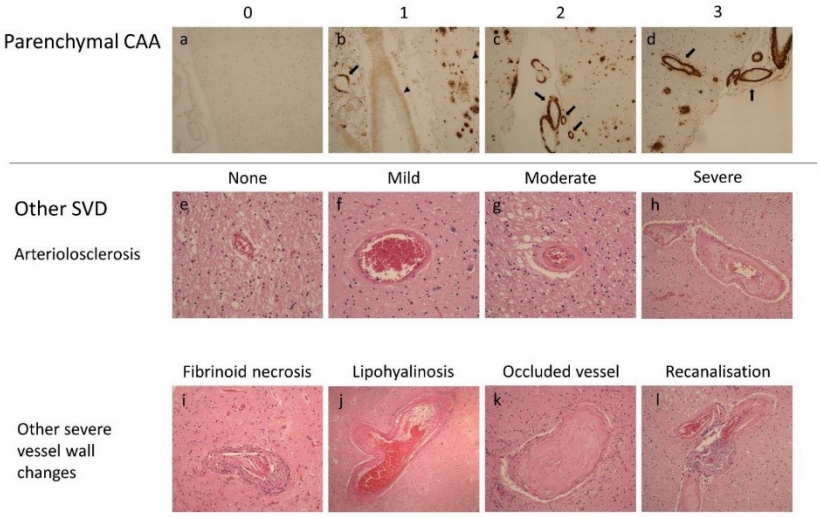
Research post-mortem

The cerebral hemispheres were sectioned in the coronal plane at 1cm intervals, the first slice taken through the mammillary bodies. The cerebellum was sectioned in the sagittal plane and the brainstem axially. Tissue samples approximately 20x20x10mm were taken from each cerebral hemisphere from: frontal parasagittal cortex (BA9); Broca's area (BA44/45); temporal tip (BA38); caudate nucleus; basal ganglia; hippocampus; thalamus; frontal, temporal, parietal and occipital white matter; cerebellum; pons and medulla. Samples were bisected in the coronal plane, one block fixed in 10% unbuffered formalin for standard histological processing and the other frozen in nitrogen vapour.[5,6]

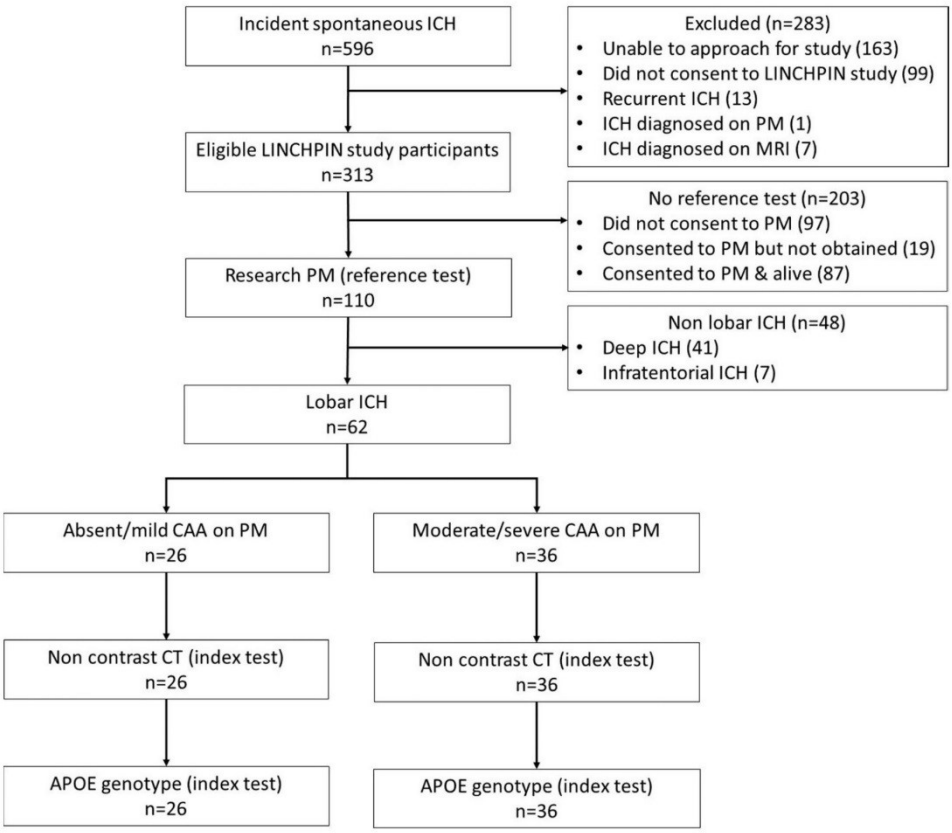
SVD assessment

We detected CAA in all cerebral and cerebellar lobes using immunohistochemistry with a monoclonal mouse antibody to human beta-amyloid, (Clone 6F/3D, Dako, Copenhagen) at a concentration of 1:100.

Example images of small vessel disease histopathological assessment grading



Flowchart of participants through the study



APOE = apolipoprotein. CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. LINCHPIN study = Lothian IntraCerebral Haemorrhage, Pathology, Imaging and Neurological Outcome study. MRI = magnetic resonance imaging. PM = research post-mortem.

Baseline characteristics of participants with lobar ICH who underwent post mortem versus those who did not

Characteristic	Post mortem (n=62)	No post mortem (n=86)	p value
Median age, years	83 (78-86)	78 (69-81)	0.00005
Male	23 (37)	36 (42)	0.56
Hypertension	42 (68)	45 (52)	0.06
Antiplatelet use at ICH	33 (53)	35 (41)	0.15
Anticoagulant use at ICH	9 (15)	14 (16)	0.75
Dementia	10 (16)	5 (6)	0.05
APOE ε2+	14 (23)	20 (33) ‡	0.19
APOE ε4+	20 (32)	25 (42)‡	0.28
Multiple ICH	9 (15)	5 (6)	0.23
Left side	32 (52)	47 (55)	0.71
ICH location*			
• Frontal	29 (47)	36 (42)	0.91
• Parietal	14 (23)	23 (27)	
• Temporal	10 (16)	13 (15)	
• Occipital	9 (15)	14 (16)	
Median ICH volume, cm³	60 (20-118)	20 (11-32)	0.000002
Strictly lobar ICH	58 (94)	85 (99)	0.16†
IV extension	31 (50)	17 (20)	0.0001
Any SAH	43 (69)	58 (67)	0.81
Subdural extension	12 (19)	14 (16)	0.63
Midline shift	39 (63)	36 (42)	0.01
Finger-like projections	14 (23)	23 (27)	0.56
Cortical involvement	56 (90)	74 (86)	0.43
Dilute/seeping	24 (39)	36 (42)	0.70
Old vascular lesion	23 (37)	29 (34)	0.67
Anterior WML			
0	10 (16)	22 (26)	0.35
1	37 (60)	48 (56)	
2	15 (24)	16 (19)	
Posterior WML			
0	13 (21)	34 (40)	0.003
1	9 (14)	21 (24)	
2	40 (65)	31 (36)	
Central atrophy			
0	19 (31)	34 (40)	0.46†
1	39 (63)	49 (57)	
2	4 (6)	3 (3)	
Cortical atrophy			
0	15 (24)	24 (28)	0.79
1	33 (53)	46 (53)	
2	14 (23)	16 (19)	
CT CAA category			
High	14 (23)	23 (27)	0.74
Intermediate	29 (47)	35 (41)	
Low	19 (31)	28 (33)	
CT & APOE CAA category			
High	24 (39)	23 (38)	0.85
Intermediate	24 (39)	21 (35)	
Low	14 (23)	16 (27)	

Data are number (%) or median (IQR). AF = atrial fibrillation. APOE ε2+ = apolipoprotein E ε2 allele present. APOE ε4+ = apolipoprotein E ε4 allele present. CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. SAH = subarachnoid haemorrhage. TIA = transient ischaemic attack. WML = white matter lucencies.* = presumed epicentre of haematoma defined by CHARTS. † = Fisher's exact test. ‡ 22 cases excluded as no APOE genotype

Intra- and inter-observer agreement for computed tomography features.

Characteristic	Frequency, n (%) (n=110)	Intra-observer agreement (95%CI) (n=110)	Inter-observer agreement (95%CI) (n=110)
Multiple ICH	12 (11)	1·00 (1·00-1·00)	0·64 (0·43-0·86)
ICH side			
Left	55 (50)	1·00 (1·00-1·00)	1·00 (1·00-1·00)
Right	51 (46)		
Central	4 (4)		
Median ICH volume, cm^3 (IQR)	31 (13-82)	0·98* (0·98-0·99)	0·96* (0·91-0·98)
Supratentorial strictly lobar ICH	58 (53)	0·98 (0·95-1·00)	0·42 (0·28-0·56)
IV extension	67 (61)	1·00 (1·00-1·00)	0·73 (0·60-0·86)
Any SAH	51 (46)	0·98 (0·95-1·00)	0·71 (0·58-0·84)
Subdural extension	12 (11)	0·96 (0·87-1·00)	0·59 (0·35-0·83)
Midline shift	66 (60)	0·75 (0·62-0·88)	0·61 (0·48-0·75)
Blood/fluid level	8 (7)	0·70 (0·41-0·98)	0·27 (-0·07-0·60)
Irregular/lobulated	69 (63)	0·68 (0·54-0·82)	0·13 (0·02-0·25)
Finger-like projections	16 (15)	0·72 (0·53-0·92)	0·60 (0·36-0·83)
Round/oval	36 (33)	0·72 (0·58-0·86)	0·05 (0·00-0·09)
Cortical involvement	60 (55)	0·98 (0·95-1·00)	0·80 (0·68-0·91)
Dilute/seeping	29 (26)	0·88 (0·78-0·98)	0·57 (0·39-0·75)
Old vascular lesion	48 (44)	0·95 (0·88-1·00)	0·56 (0·41-0·72)
Anterior WML			
0	14 (13)	0·76† (0·65-0·88)	0·54† (0·40-0·67)
1	66 (60)		
2	30 (27)		
Posterior WML			
0	27 (25)	0·75† (0·66-0·84)	0·52† (0·40-0·64)
1	20 (18)		
2	63 (57)		
Central atrophy			
0	24 (22)	0·70† (0·58-0·82)	0·51† (0·37-0·64)
1	66 (60)		
2	20 (18)		
Cortical atrophy			
0	19 (17)	0·65† (0·53-0·78)	0·55† (0·41-0·69)
1	64 (58)		
2	27 (25)		

Agreement assessed with un-weighted Cohen's kappa, linear-weighted kappa†, or intraclass correlation coefficient*. ICH = intracerebral haemorrhage. Cohen suggested the Kappa result be interpreted as follows: values ≤ 0 as indicating no agreement and 0.01–0.20 as none to slight, 0.21–0.40 as fair, 0.41– 0.60 as moderate, 0.61–0.80 as substantial, and 0.81–1.00 as almost perfect agreement. IV = intraventricular. SAH = subarachnoid haemorrhage. WML = white matter lesion.

Baseline characteristics of all ICH, stratified by location

	All ICH (n=110)	Lobar ICH (n=62)	Non-lobar ICH (n=48)
Median age at ICH, years	83 (76-87)	83 (78-86)	82 (75-87)
Male	49 (45)	23 (37)	26 (54)
Median time from ICH to CT, hours	5 (3-18)	6 (3-29)	4 (3-15)
Median time from CT to post-mortem, days	11 (5-80)	11 (6-134)	9 (5-23)
Previous ischaemic stroke/TIA	23 (21)	12 (19)	11 (23)
Coronary artery disease	25 (23)	16 (26)	9 (19)
Atrial fibrillation	31 (28)	19 (31)	12 (25)
Diabetes	14 (13)	6 (10)	8 (17)
Hypertension	77 (70)	42 (68)	35 (73)
Hyperlipidaemia	15 (14)	8 (13)	7 (15)
Antiplatelet use at ICH	53 (48)	33 (53)	20 (42)
Anticoagulant use at ICH	20 (18)	9 (15)	11 (23)
Dementia before ICH	18 (16)	10 (16)	8 (17)
APOE ε2+	21 (19)	14 (23)	7 (15)
APOE ε4+	34 (31)	20 (32)	14 (29)
ICH location*			
• Lobar	62 (56)	62 (100)	
○ Frontal	29 (26)	29 (47)	
○ Parietal	14 (13)	14 (23)	
○ Temporal	10 (9)	10 (16)	
○ Occipital	9 (8)	9 (15)	
• Deep	41 (37)		41 (85)
○ Basal ganglia	22 (20)		22 (46)
○ Thalamic	19 (17)		19 (40)
• Infratentorial	7 (6)		7 (15)
○ Brainstem	4 (4)		4 (8)
○ Cerebellum	3 (3)		3 (6)

Data are number (%) or median (IQR). AF = atrial fibrillation. APOE ε2+= apolipoprotein E ε2. allele present. APOE ε4+ = apolipoprotein E ε4 allele present. CT = computed tomography. ICH = intracerebral haemorrhage. TIA = transient ischaemic attack. * = presumed epicentre of haematoma defined by CHARTS[4]

Distribution of cerebral small vessel disease sub-types by ICH location

	Non-lobar ICH (n=48)		Lobar ICH (n=62)	
Pathology Category	*CAA absent*	*CAA present*	*CAA absent*	*CAA present*
Other SVD absent	0 (0)	0 (0)	0 (0)	0 (0)
Other SVD present	32 (67)	16 (33)	16 (26)	46 (74)
Pathology Category	*CAA absent/mild*	*CAA moderate/severe*	*CAA absent/mild*	*CAA moderate/severe*
Other SVD absent/mild	0 (0)	0 (0)	2 (3)	10 (16)
Other SVD moderate/severe	42 (88)	6 (13)	24 (39)	26 (42)

Data are number (%). CAA = cerebral amyloid angiopathy. ICH = intracerebral haemorrhage. SVD = small vessel disease

Logistic regression model with Firth correction fitted in participants with lobar ICH associated with moderate/severe CAA, excluding 9 cases taking oral anticoagulants at the time of ICH

	β Coefficient (standard error)	Odds ratio (95%CI)	p value
Intercept	-2·43 (0·92)		0·009
APOE ε4 +	2·77 (1·00)	15·97 (2·93-587·82)	0·006
Subarachnoid haemorrhage	2·18 (0·97)	8·89 (1·69-277·39)	0·02
Finger-like projections	3·09 (1·60)	21·97 (2·19-∞)	0·05

APOE ε4− = apolipoprotein E ε4 allele present.

Performance measures of the diagnostic prediction model in the development dataset (n=62) and following internal validation using the same dataset (n=62; 2,000 bootstrap samples)

	Development	Internal validation
Overall		
Brier score	0·11	0·12
R^2 (Nagelkerke)	0·57	0·51
Akaike information criterion	49·90	55·90
Discrimination		
c statistic	0·92	0·91
Discrimination slope	0·52	0·50
Calibration		
Hosmer-Lemeshow test	$\chi^2 = 0·55$, p = 0·76	$\chi^2 = 2·79$, p = 0·25

Predicted and observed frequencies, and categorisation of the probability of lobar ICH associated with moderate/severe CAA according to the three predictor variables

Predictors present			Predicted risk of moderate/severe CAA, %	Observed frequency of moderate/severe CAA (%) [95%CI]			Moderate/severe CAA probability
Subarachnoid haemorrhage	APOE ε4+	Finger-like projections					
-	-	-	7	0/14	(0)	[0-22%]	Low
+	-	-	44	9/19	(47)	[27-68%]	Medium
-	+	-	64	4/5	(80)	[38-99%]	
+	+	-	95	9/10	(90)	[60-99%]	
+	-	+	95	9/9	(100)	[70-100%]	High
+	+	+	100	5/5	(100)	[57-100%]	

Cross tabulations of the Edinburgh CT and genetic diagnostic criteria for lobar ICH associated with moderate/severe CAA against the reference standard

Diagnostic criteria (index test)	Reference standard		
Subarachnoid haemorrhage or APOE ε4+	**Moderate/severe CAA at post-mortem**		
	Present	Absent	Total
Positive	36	12	48
Negative	0	14	14
Total	36	26	62

Diagnostic criteria (index test)	Reference standard		
Subarachnoid haemorrhage and (APOE ε4+ or finger-like projections)	**Moderate/severe CAA at post-mortem**		
	Present	Absent	Total
Positive	23	1	24
Negative	13	25	38
Total	36	26	62

APOE = apolipoprotein. CAA = cerebral amyloid angiopathy. CT = computed tomography.

Diagnostic test accuracy statistics for the two sets of Edinburgh CT and genetic diagnostic criteria for lobar ICH associated with moderate/severe CAA

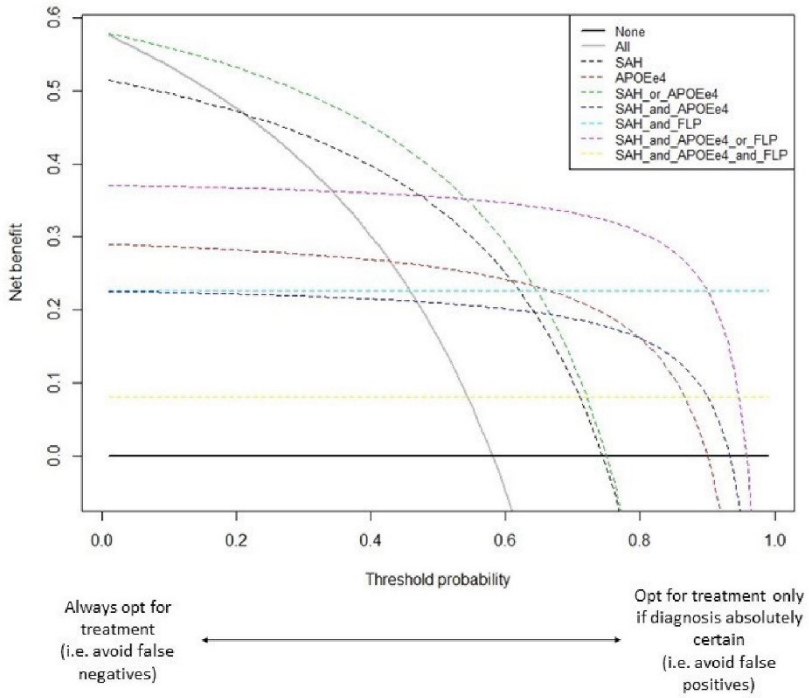
	Edinburgh CT and genetic diagnostic criteria	
	Subarachnoid haemorrhage or APOE ε4+	Subarachnoid haemorrhage and (APOE ε4+ or finger-like projections)
Sensitivity	100 (88-100)	64 (46-79)
Specificity	54 (34-73)	96 (78-100)
Positive likelihood ratio	2·2 (1·4-3·3)	16·6 (2·4-115·3)
Negative likelihood ratio	0 (0-NaN)	0·4 (0·2-0·6)
Positive predictive value	75 (60-86)	96 (77-100)
Negative predictive value	100 (73-100)	66 (49-80)
Youden's Index	0·54	0·60

Data are percentage or ratio (95% confidence interval). APOE = apolipoprotein. CAA = cerebral amyloid angiopathy. CT = computed tomography. NaN = not a number – calculation cannot be performed because one of the values includes a zero

Decision curves of predictions and classifications of moderate/severe CAA in participants with lobar ICH using fixed cut-off points from the CT and APOE genotype prediction model to assign patients as positive or negative for moderate/severe CAA.

The threshold probability is the level of diagnostic certainty above which a patient or clinician would choose to be treated. Equally, it could be used by a researcher to identify patients eligible for inclusion in a randomised controlled trial or identify cases and controls for a case-control study. The threshold probability is low in situations where we want to avoid false negatives (e.g. when trying to rule out CAA-associated lobar ICH) and high when false positives are to be avoid (e.g. when trying to rule in CAA-associated lobar ICH). Net benefit is the difference between those expected to benefit (true positives identified using the strategy – expected benefit) and those expected to be harmed (false positives identified using the strategy multiplied by a weighting factor based on the threshold probability – expected harm). The curves which maximise net benefit represent the optimal strategy for the associated threshold probabilities. The solid black line indicates a policy of treating no one, the grey line a policy of treating all.

The combination of subarachnoid haemorrhage or APOEε4 is the best diagnostic strategy for low threshold probabilities (0-0.55), where harm of unnecessary treatment is limited and false negatives avoided (i.e. useful for ruling CAA-associated lobar ICH out). For high threshold probabilities (0.55-0.90), where there is harm of overtreatment and false positives should be avoided, the criteria of subarachnoid haemorrhage AND (APOE ε4 OR Finger-like projections) maximises net benefit (i.e. for ruling CAA-associated lobar ICH in).



APOE ε4 = apolipoprotein E ε4 allele present. FLP = finger-like projection. SAH = subarachnoid haemorrhage

Logistic regression model using PMW ratings for CT-based features and APOE genotype, with Firth correction fitted in participants with lobar ICH associated with moderate/severe CAA.

	β Coefficient (standard error)	Odds ratio (95%CI)	p value
Intercept	-3·39 (1.46)		0·02
APOE ε4 +	4·18 (1.49)	65 (7-∞)	0·005
Subarachnoid haemorrhage	3·09 (1.47)	22 (3-∞)	0·04
Finger-like projections	3·01 (1.61)	20 (2-∞)	0·06

APOE ε4 + = apolipoprotein E ε4 allele present.

This model calculates the predicted probability of moderate/severe CAA as follows:

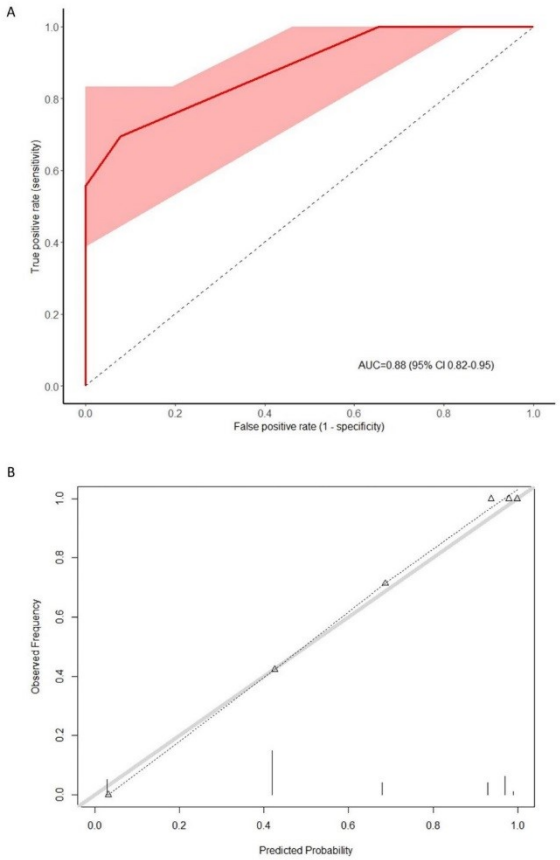
Predicted probability = $1/(1 + \exp^{-\text{risk score}})$

Risk score = -3.39 + 4.18 x (APOEe4+) + 3.09 x (subarachnoid haemorrhage) + 3.01 x (finger-like projections)

The predictor values are one when present and zero when absent.

Discrimination and calibration measures of CT and APOE diagnostic prediction model performance using PMW CT ratings

A. Receiver operating characteristic (ROC) curve for predicted probability of moderate/severe CAA. The area under the curve (AUC) is equivalent to the c statistic. The shaded area represents the 95% confidence interval of the AUC based on 2,000 bootstrap replicates. The dotted line indicates a non-informative AUC of 0·50 for comparison. B. Calibration plot of predicted probability versus observed frequency of moderate/severe CAA. The grey line indicates perfect calibration, the model's calibration is shown by the dotted line. The triangles represent the six different moderate or severe CAA risk groups produced by the prediction model. The vertical lines along the x axis represent the frequency and distribution of model predicted probabilities.



Performance measures of CT and APOE diagnostic prediction model using PMW CT ratings in the development dataset (n = 62) and following internal validation using the same dataset (n = 62; 2,000 bootstrap samples).

	Development	Internal validation
Overall		
Brier score	0.13	0.14
R^2 (Nagelkerke)	0.51	0.47
Akaike information criterion	53.78	57.86
Discrimination		
c statistic	0.88	0.88
Discrimination slope	0.45	0.43
Calibration		
Hosmer-Lemeshow test	$\chi^2 = 0{\cdot}74$, $p = 0{\cdot}86$	$\chi^2 = 2.01$, $p = 0{\cdot}37$

Risk categories for lobar ICH associated with moderate/severe CAA using CT and APOE diagnostic criteria using PMW CT ratings

APOE ε4+ = apolipoprotein E ε4 allele present. CAA = cerebral amyloid angiopathy

Predictors present			Predicted risk of moderate/severe CAA, %	Observed frequency of moderate/severe CAA (%) [95%CI]			Moderate/ severe CAA probability
Subarachnoid haemorrhage	APOE ε4+	Finger-like projections					
-	-	-	3	0/9	(0)	[0-30%]	Low
+	-	-	43	11/26	(42)	[26-61%]	Medium
-	+	-	69	5/7	(71)	[36-92%]	
+	+	-	98	11/11	(100)	[74-100%]	
+	-	+	94	7/7	(100)	[65-100%]	High
+	+	+	100	2/2	(100)	[34-100%]	

Cross tabulations of the Edinburgh CT and genetic diagnostic criteria for lobar ICH associated with moderate/severe CAA using PMW CT ratings against the reference standard

Diagnostic criteria (index test)	Reference standard		
Subarachnoid haemorrhage or APOE ε4+	**Moderate/severe CAA at post-mortem**		
	Present	Absent	Total
Positive	36	17	53
Negative	0	9	9
Total	36	26	62
Diagnostic criteria (index test)	**Reference standard**		
Subarachnoid haemorrhage and (APOE ε4+ or finger-like projections)	**Moderate/severe CAA at post-mortem**		
	Present	Absent	Total
Positive	20	0	20
Negative	16	26	42
Total	36	26	62

APOE ε4+ = apolipoprotein E ε4 allele present. CAA = cerebral amyloid angiopathy

Diagnostic test accuracy statistics for the Edinburgh CT and genetic diagnostic criteria for lobar ICH associated with moderate/severe CAA using PMW CT ratings

	Edinburgh CT and genetic diagnostic criteria			
	Subarachnoid haemorrhage or APOE ε4+		Subarachnoid haemorrhage and (APOE ε4+ or finger-like projections)	
Sensitivity	100	(88-100)	56	(38-72)
Specificity	35	(18-56)	100	(84-100)
Positive likelihood ratio	1.5	(1.2-2.0)	∞	(NaN-∞)
Negative likelihood ratio	0	(0-NaN)	0.4	(0.3-0.6)
Positive predictive value	68	(54-80)	100	(80-100)
Negative predictive value	100	(63-100)	62	(46-76)
Youden's Index	0.35		0.56	

Data are percentage or ratio (95% confidence interval). NaN = not a number – calculation cannot be performed because one of the values includes a zero.

Comparison of the Edinburgh CT and genetic criteria with the modified Boston criteria in participants with lobar ICH who underwent MRI during life

Age at ICH	Modified Boston MRI classification	Edinburgh CT and genetic classification	Pathological CAA grade
79	Probable	Iligh	Severe
76	Probable	High	Moderate
72	Probable	Iligh	Moderate
84	Probable	Intermediate	Severe
90	Probable	Intermediate	Mild
83	Probable	Intermediate	Absent
83	Probable	Low	Absent

Logistic regression model using only CT-based features without APOE genotype, with Firth correction fitted in participants with lobar ICH associated with moderate/severe CAA.

	β Coefficient (standard error)	Odds ratio (95%CI)	p value
Intercept	-1·24 (0·55)		0·02
Subarachnoid haemorrhage	1·71 (0·67)	5·54 (1·63-26·09)	0·01
Finger-like projections	2·89 (1·54)	18·03 (2·04-∞)	0·06

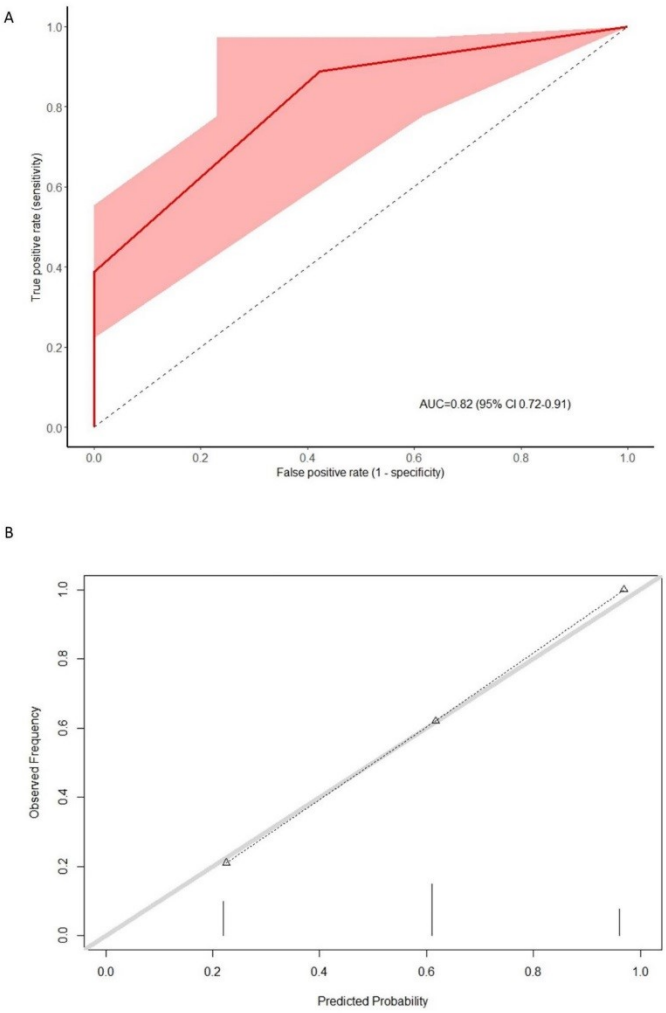
This model calculates the predicted probability of moderate/severe CAA as follows:

Predicted probability = $1/(1 + \exp^{-\text{risk score}})$

Risk score = -1·24 + 1·71 x (subarachnoid haemorrhage) + 2·89 x (finger-like projections)

The predictor values are one when present and zero when absent.

Discrimination and calibration measures of CT-based diagnostic prediction model without APOE genotype
A. Receiver operating characteristic (ROC) curve for predicted probability of moderate/severe CAA. The area under the curve (AUC) is equivalent to the c statistic. The shaded area represents the 95% confidence interval of the AUC based on 2,000 bootstrap replicates. The dotted line indicates a non-informative AUC of 0·50 for comparison. B. Calibration plot of predicted probability versus observed frequency of moderate/severe CAA. The grey line indicates perfect calibration, the model's calibration is shown by the dotted line. The triangles represent the three different moderate or severe CAA risk groups produced by the prediction model. The vertical lines along the x axis represent the frequency and distribution of model predicted probabilities.



Performance measures of CT-based diagnostic prediction model without APOE genotype in the development dataset (n = 62) and following internal validation using the same dataset (n = 62; 2,000 bootstrap samples).

	Development	Internal validation
Overall		
Brier score	0·16	0·17
R^2 (Nagelkerke)	0·31	0·28
Akaike information criterion	65·03	68·90
Discrimination		
c statistic	0·82	0·82
Discrimination slope	0·32	0·31
Calibration		
Hosmer-Lemeshow test	$\chi^2 = 0{\cdot}48$, p = 0·92	$\chi^2 = 1{\cdot}35$, p = 0·72

Risk categories for lobar ICH associated with moderate/severe CAA using CT-based diagnostic criteria without APOE genotype

Predictors present		Predicted risk of moderate/severe CAA, %	Observed frequency of moderate/severe CAA (%) [95% CI]		Moderate/severe CAA probability
Subarachnoid haemorrhage	Finger-like projections				
-	-	23	4/19 (21)	[9-43]	Low
+	-	62	18/29 (62)	[44-77]	Medium
+	+	97	14/14 (100)	[78-100]	High

Cross tabulations of the index test results against the reference standard using the simplified Edinburgh (CT-only) CAA-associated lobar ICH diagnostic criteria

Diagnostic criteria (index test) Subarachnoid haemorrhage	Reference standard Moderate/severe CAA at post mortem		
	Positive	Negative	Total
Positive	32	11	43
Negative	4	15	19
Total	36	26	62
Diagnostic criteria (index test)	**Reference standard**		
Subarachnoid haemorrhage and finger-like projections	**Moderate/severe CAA at post mortem**		
	Positive	Negative	Total
Positive	14	0	14
Negative	22	26	48
Total	36	26	62

Diagnostic test accuracy statistics for the simplified Edinburgh (CT-only) CAA-associated lobar ICH diagnostic criteria without APOE genotype

	Diagnostic criteria	
	Subarachnoid haemorrhage	Subarachnoid haemorrhage and finger-like projections
Sensitivity	89 (73-96)	39 (24-56)
Specificity	58 (37-76)	100 (84-100)
Positive likelihood ratio	2·1 (1·3-3·3)	Inf (NaN-∞)
Negative likelihood ratio	0·2 (0·1-0·5)	0·6 (0·5-0·8)
Positive predictive value	74 (59-86)	100 (73-100)
Negative predictive value	79 (54-93)	54 (39-68)
Youden's Index	0·47	0·39

Data are percentage or ratio (95% confidence interval). NaN = not a number – calculation cannot be performed because one of the values includes a zero.

Unique MRC Brain Brank Network (BBN) numbers of included participants

BBN_7268	BBN_2561	BBN_14393	BBN_19602	BBN_24323
BBN_2519	BBN001.26127	BBN_19993	BBN_19601	BBN_24343
BBN_2514	BBN_2563	BBN_9507	BBN_19599	BBN_24528
BBN_15254	BBN_2570	BBN001.26492	BBN_19600	BBN_25059
BBN_2554	BBN_2573	BBN_7276	BBN_19598	BBN_24670
BBN_2524	BBN_15813	BBN_9504	BBN_19364	BBN_24531
BBN001.26092	BBN_4173	BBN_9502	BBN_19596	BBN_25060
BBN_2583	BBN_20599	BBN_9506	BBN_22228	BBN_25057
BBN_2526	BBN_2578	BBN_15215	BBN_20994	BBN_25058
BBN_3776	BBN_2582	BBN20615	BBN_20611	BBN001.26493
BBN_2535	BBN_20602	BBN_15815	BBN_20603	BBN001.26394
BBN_3769	BBN_3763	BBN_24305	BBN_22224	BBN001.26491
BBN_2527	BBN_3764	BBN_15224	BBN_24302	BBN001.26490
BBN_2539	BBN_3766	BBN_20992	BBN_22227	BBN001.26496
BBN_2543	BBN_3777	BBN001.26307	BBN_22223	BBN001.26499
BBN_2574	BBN_3775	BBN_15812	BBN_22226	BBN001.26719
BBN_2545	BBN_19994	BBN_15814	BBN_22626	BBN001.26722
BBN_2544	BBN_3781	BBN_15811	BBN_22627	BBN001.28404
BBN_2546	BBN_3782	BBN_19605	BBN_23394	BBN001.28405
BBN_19597	BBN_7627	BBN_19606	BBN_24303	BBN001.28408
BBN_2548	BBN_3787	BBN_19604	BBN_24304	BBN001.28411
BBN_2549	BBN_4167	BBN_19603	BBN_24306	BBN001.28416

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7.2 Conclusion

The CT-only and CT-APOE diagnostic prediction models showed excellent discrimination and were well calibrated for CAA-associated lobar ICH. The related Edinburgh criteria were able to accurately rule in or rule out CAA-associated lobar ICH. These diagnostic models and simple diagnostic criteria may be useful in routine clinical practice for diagnosing or ruling out CAA-associated lobar ICH, however their accuracy needs to be assessed in large, rigorous external validation studies.

There were no participants in the development study with finger-like projections but absent subarachnoid haemorrhage. Based on the logistic regression models, this combination of features is classified as intermediate CAA probability. However, I was unable to assess the models' calibration for this potential group. The diagnostic accuracy of the Edinburgh criteria for this combination of predictors should be assessed in larger external validation studies.

I did not include an age cut-off as part of the Edinburgh criteria, as age was similar between participants with absent or mild CAA (median 84 years; IQR 78-88) and those with moderate or severe CAA (median 82 years; IQR 79-85). However, CAA is an age-related condition and unlikely to occur in those younger than 50 years. Indeed, although I included all adults with first-ever spontaneous lobar ICH in my development study, the youngest participant with lobar ICH was 56 years, and the youngest with CAA-associated lobar ICH was 67 years. The validity of the Edinburgh criteria in lobar ICH patients younger than this is unclear, and should be specifically addressed in external validation studies.

Reliable implementation of diagnostic criteria is a key determinate of their clinical utility. In this development study there was moderate inter-rater agreement for finger-like projections and good agreement for subarachnoid haemorrhage. To improve the inter-rate agreement for these key imaging features, I have developed a series of online training materials for implementing the Edinburgh criteria.[330] These training materials are

available to any clinician or researcher. They include a series of test cases, which will allow me to assess the inter-rater agreement of the Edinburgh criteria across a wide range of raters of differing background and experience.

The prognostic value of the Edinburgh criteria, for the risk of recurrent ICH and the development of post-stroke dementia in ICH survivors will also be important to assess.

Chapter 8 External validation studies of the Edinburgh CT-only and CT-APOE diagnostic models and criteria for CAA-associated lobar ICH

8.1 Introduction

The diagnostic models for CAA-associated lobar ICH which I have developed using non-contrast CT brain features, and APOE ε4 genotype when available, showed excellent discrimination and good calibration in the development setting.[229] The models had little optimism after internal validation, confirming their reproducibility and validity in the development setting. The related Edinburgh diagnostic criteria were able to rule out or rule in CAA. However, external validation is required to determine whether the diagnostic models and criteria are reliable in other settings.[331]

External validation is the process of evaluating prediction models in datasets not used in model development.[331] External validation can be temporal (more recently affected individuals from the same geographical location as the development cohort), geographic (individuals from geographically distinct areas compared with the development cohort) or mixed. It permits the assessment of model performance in new individuals, allowing more accurate quantification of model optimism. Also, the use of plausibly similar individuals from other geographical regions and with different disease severity (different case-mix) allows the generalisability of the model to be determined.

The diagnostic models I have developed used participants from the NHS Lothian Health Board region of Scotland who had died after ICH and who underwent a research autopsy. As a result, the participants tended to be white British. Also, they were older, had larger ICHs, more advanced CT SVD biomarkers, and usually died soon after their ICH compared with those not

included in the study.[229] Therefore external validation in other geographic areas and in less severe ICH cases is essential to assess the models' generalisability.

8.2 Aims

I aimed to perform international, multi-centre external validation studies of the Edinburgh CT-only and CT and APOE diagnostic models for CAA-associated lobar ICH, and assess the diagnostic accuracy of the related Edinburgh criteria against a histopathological reference standard. I wanted to include participants from different geographic regions to assess the generalisability. Also, I aimed to allow brain biopsy, as well as autopsy, as the histopathological reference standard to determine whether the models and criteria are valid in less severe ICH.

8.3 Methods

I performed an international, multicentre external validation study of the Edinburgh diagnostic models and criteria according to a pre-specified study protocols (Appendix 3). I performed and reported the study according to the TRIPOD and STARD guidelines.[173, 332]

8.3.1 Sources of data

I identified potential collaborators using two approaches.

Firstly, I contacted the International CAA Association, which is coordinating an update study of the modified Boston criteria. Through this, they have identified research groups with imaging and subsequent histopathology for CAA in spontaneous ICH. Dr Andreas Charidimou, who is coordinating this project, reviewed all groups invited to join the Boston criteria update study against the eligibility criteria in my study protocol (Section 8.3.2 and Appendix 3). He identified 14 groups with potentially relevant data for my study.

Secondly, I used our recent systematic review of the imaging features of CAA-associated ICH to identify groups who had published studies comparing CT imaging against histopathological assessment of CAA in ICH.[166] I re-ran the search strategy from this systematic review on 01/06/2018 to identify relevant new studies and abstracts. I reviewed all the papers and abstracts according to the eligibility criteria (Section 8.3.2 and Appendix 3) and identified 11 groups with potentially relevant data.

Six groups were identified by both the International CAA Association and by my systematic review (Edinburgh, Boston, Lille, Calgary, Porto and Kanazawa). Therefore in total, I identified 19 individual research groups with potentially relevant data.

In June 2018, I emailed the corresponding author from all 19 individual research groups inviting them to collaborate in this study (Appendix 4). I sent a reminder email in August 2018 to those who had not replied to my initial invitation. Twelve groups responded to my email invitation. Nine (47% of the studies) were able to contribute data from their cohorts (Table 8.1), five of which were identified by the International CAA Association and four by both the International CAA Association and my systematic review.

8.3.2 Study design and participants

I included data from all nine cohorts. Details of the study design, study setting and location of the centres are included in Table 8.1. The study design varied between collaborating centres, although most were hospital-based case-series or cohort studies.

Table 8.1 Characteristics of included cohorts.

Study group	Study recruitment start/end date	Setting	Region/centre	Sampling approach	Case identification	Reason for performing			Number of included participants	
						APOE	Brain biopsy	Autopsy	CT-only	CT & APOE
Edinburgh	11/02/2016 to 28/09/2018	Community-based	NHS Lothian Health Board, UK	Consecutive	Prospective	Offered to all	N/A	Offered to all	8	7
Magdeberg	20/01/2002 to 24/09/2018	Hospital-based	University Hospital Magdeberg, Germany	Consecutive	Retrospective	Participation in other studies	Offered to all	Offered to all	27	1
Hungary	01/01/2013 to 10/08/2018	Hospital-based	University of Szeged, Hungary	Consecutive	Prospective	Offered to all	Caring Physician decision	Caring Physician decision	3	0
Reggio Emilia	01/01/2006 to 30/06/2018	Hospital-based	Azienda Unità Sanitaria Locale, Reggio Emilia, Italy	Consecutive	Retrospective prior to January 2014 Prospective since January 2014	Caring Physician decision	Caring Physician decision	N/A	11	4
Porto	01/01/2002 to 31/07/2018	Hospital-based	Centro Hospitalar Universitário do Porto, Portugal	Consecutive	Retrospective	N/A	Caring Physician decision	N/A	1	0

Study group	Study recruitment start/end date	Setting	Region/centre	Sampling approach	Case identification	Reason for performing			Number of included participants	
						APOE	Brain biopsy	Autopsy	CT-only	CT & APOE
London	01/01/2002 to 31/07/2018	Hospital-based	University College Hospital and The National Hospital, Queen Square, London, UK	Convenience	Retrospective	N/A	Caring Physician decision	N/A	1	0
Lille	03/11/2004 to 29/03/2009	Hospital-based	Lille University Hospital, France	Consecutive	Prospective	N/A	Hospital policy	Caring Physician decision	7	0
Munich	01/03/2001 to 31/07/2018	Hospital-based	Ludwig Maximilians University of Munich Hospital, Germany	Consecutive	Retrospective	Performed on biopsy/ autopsy tissue if sufficient tissue available	Caring Physician decision	Caring Physician decision	14	10
Boston	1999 to 2018	Hospital-based	Massachusetts General Hospital, Boston, USA	Consecutive	Prospective	Offered to all	Routine clinical care	Routine clinical care	74	43

APOE = apolipoprotein E. CT = computed tomography

I included adult patients (aged ≥16 years) with a first-ever lobar ICH diagnosed by non-contrast brain CT who had a subsequent histopathological assessment for CAA.

I defined lobar ICH as described in Section 2.1.5.[190]

I excluded patients with exclusively extra-axial intracranial haemorrhage, non-lobar ICH, ICH secondary to an underlying cause other than SVDs, and those without diagnostic quality non-contrast brain CT and histopathological samples for CAA assessment.

These inclusion and exclusion criteria were the same as those I used for developing the Edinburgh diagnostic criteria.[229]

In addition to the above, I excluded all participants included in the development of the Edinburgh diagnostic criteria (i.e. Edinburgh LINCHPIN cohort with ICH onset date between 1st June 2010 and 10th February 2016).[229]

For the external validation of the CT and genetic model and criteria, I excluded all cases without APOE genotyping.

8.3.3 Baseline data collection

Collaborators collected demographics, the presence of relevant co-morbidities and medication use at the time of ICH by interviewing patients and/or reviewing medical records (Appendix 3).

8.3.4 Index tests

I reformatted non-contrast brain CT volume datasets into standard axial, coronal and sagittal planes as described in Section 2.2.1.2.[229] I assessed all available brain CT planes. When a volume dataset was not available, I reviewed the axial plane acquisition.

I assessed the presence, number and location of acute ICH. I calculated the volume of the largest ICH using a modified ABC/2 approach as described in Section 2.2.1.3.[191] I rated the presence or absence of extra-axial haemorrhage (subarachnoid, subdural or intra-ventricular spaces) and finger-

like projections arising from the largest haematoma. I defined finger-like projections in the same way as I did in the development study as elongated extensions arising from the haematoma, longer than they are wide, regardless of whether they extended to the cortex or not.[229] I rated finger-like projections from the largest ICH if multiple acute ICHs were present on the CT scan.

I developed and undertook online training materials for implementing the Edinburgh criteria before assessing the brain CT images.[330] I performed brain CT assessments masked to clinical, genetic and pathological data.

Collaborators performed APOE genotyping on DNA extracted from peripheral blood or brain tissue using standard techniques, masked to radiological, clinical and pathological data. I defined APOE ε2 and APOE ε4 possession if a participant had at least one ε2 or ε4 allele respectively, as described in Chapter 7.[229]

I used the same definitions for predictors (subarachnoid haemorrhage, finger-like projections and APOE ε4 possession) as I did during the development of the criteria.

8.3.5 Reference standard

The outcome of interest was CAA-associated lobar ICH defined by histopathological assessment of brain biopsy or autopsy material using Congo red and/or β-amyloid immunohistochemistry. I defined CAA-associated lobar ICH when the severity of CAA in any tissue sample was ≥2 on the Vonsattel scale.[253]

The sources of tissue and reference standard definition differed from the approach I took in the development of the Edinburgh diagnostic models and criteria.

In the development study, I restricted the reference standard to research autopsy. In this external validation study, I included both brain biopsy and autopsy as sources of pathological tissue. I did this because I wanted to assess if the Edinburgh diagnostic models and criteria were applicable in a

wider range of ICH severity, rather than predominantly acutely fatal ICH which I used in the development study. While brain biopsy may be more prone to sampling error in comparison to autopsy, I have shown in Section 4.4.4 that simulated brain biopsies from the lobe affected by ICH have excellent sensitivity and good specificity for global CAA severity identified on full autopsy. These results are similar to a previously published simulated biopsy study.[164]

In the development study, a single neuropathologist assessed CAA severity. In line with the International CAA Association's external validation and update of the modified Boston criteria, the histopathological reference standard in this external validation study was assessed locally by experienced neuropathologists. Sending pathological tissue to a central centre for rating by a single neuropathologist was not practical for logistical and regulatory approval reasons in this multi-centre study.

In the development study, the CAA severity was assessed using a consensus scale.[36] In this external validation study, the severity of CAA was evaluated using the Vonsattel scale[253] for two reasons. Firstly, the Vonsattel scale was used as the reference standard in the update of the modified Boston criteria study. Many of the centres involved in that study were included in this external validation study. Therefore, I wanted to harmonise reference standards to minimise additional workload for collaborators. Secondly, the Vonsattel scale has been in use for over 25 years, making it familiar to many neuropathologists.

I chose a cut-off of ≥2 on the Vonsattal scale (complete replacement of the media by β-amyloid) to define CAA-associated ICH as this definition is the closest to the cut off I used on the CAA consensus scale during the development study (some circumferential β-amyloid), and resulted in the best sensitivity and specificity against the consensus scale ratings (Section 4.4.4).[36]

The histopathological assessments were performed masked to CT, clinical and genetic data.

8.3.6 Sample size

I did not perform a formal sample size calculation as there is no generally accepted approach for this in validation studies.[333] General guidance is to include at least 100 cases and 100 controls to be able to detect modest changes in model performance measures.[334, 335] I included all available data from the collaborators I had identified through the International CAA association and through systematically reviewing the literature to maximise power.

8.3.7 Missing data

I excluded any cases with missing predictors as specified in the study protocol. It is difficult to assess the presence of subarachnoid haemorrhage or finger-like projections if an acute ICH is not visible on the diagnostic CT scan. I did not think it was appropriate to impute these data as they form the main predictors in the diagnostic models. Similarly, APOE ε4 is one of only three predictors in the CT and genetic diagnostic model, so I decided not to impute the missing values. All cases had the reference standard available.

8.3.8 Statistical analysis

I compared the frequency of clinical, genetic, and CT characteristics in participants with and without CAA-associated lobar ICH according to the reference standard using χ^2 test (or Fisher's exact test, where appropriate) for categorical variables and the Mann-Whitney U test for non-normally distributed continuous variables.

To assess the performance of the Edinburgh diagnostic models, I first calculated the risk score of moderate/severe CAA using the regression equations for the CT-only and CT and APOE models (Chapter 7, [229]) as follows:

> CT-only model risk score = 1·71 x subarachnoid haemorrhage + 2·89 x finger-like projections -1·24
>
> CT and APOE model risk score = 3.11 x APOE4 possession + 2.31 x subarachnoid haemorrhage + 3.20 x finger-like projections -2.55

The predictor values are one when present and zero when absent.

I then calculated the predicted probability of moderate/severe CAA using the following formula:

$$\text{Predicted probability} = 1/(1 + \exp^{-\text{risk score}})$$

I evaluated model calibration using calibration plots to assess the agreement between the model predicted and the observed frequency of CAA-associated ICH and calculated the intercept and the calibration slope. The intercept relates to calibration-in-the-large (a comparison of the mean of all predicted risks and the mean observed risk).[336] The calibration slope is related to shrinkage of regression coefficients during model development. A perfectly calibrated model will have an intercept of 0 and a calibration slope of 1. Miscalibration results in an intercept either above or below 0 and a calibration slope less than 1. I evaluated model discrimination using ROC curves and quantified the performance of the models using the concordance (c) statistic. I assessed the net benefit of the model using decision curve analysis.[337]

To determine the performance of the Edinburgh diagnostic criteria I classified participants as low, intermediate or high risk of CAA-associated lobar ICH using the cut-offs defined in the development study (Chapter 7).[229] I then assessed diagnostic accuracy statistics (sensitivity, specificity, likelihood ratios and predictive values) between low versus intermediate/high risk (rule out criteria) and high versus low/intermediate risk groups (rule in criteria).

I performed a pre-specified sensitivity analysis of the CT-only diagnostic model and criteria's performance stratifying the reference standard according to the source of tissue (brain biopsy versus autopsy) given the differing sensitivity and specificity of these approaches for CAA (Section 4.4.4).[164] I also performed an exploratory sensitivity analysis by stratifying participants by ICH volume (less than median ICH volume (56 ml) versus greater than or equal to the median ICH volume) and time between diagnostic CT and tissue sampling based on my review of false negative cases.

I did not perform sensitivity analyses of the CT and APOE diagnostic model and criteria due to the small sample size in this part of the study.

8.4 Results

8.4.1 External validation of the Edinburgh CT-only diagnostic model and criteria

8.4.1.1 Flow of participants

There were 164 potentially eligible participants identified by the nine collaborating groups. After unavoidable exclusions, I included 146 (Figure 8.1).

8.4.1.2 Comparison of participants included in the external validation study with those in the development study

Participants in the external validation cohort were younger compared with those in the development cohort (Table 8.2). Pre-ICH history of ischaemic stroke and of atrial fibrillation were less common in the external validation cohort than in the development cohort, whilst hyperlipidaemia was more common in the external validation cohort. The frequency of other comorbidities was similar between the cohorts. Fewer participants in the external validation cohort were taking an antiplatelet drug at the time of ICH. Admission GCS and ICH volume were similar between the external validation and development cohorts (Table 8.3).

Subarachnoid haemorrhage and finger-like projections were more frequent in the external validation cohort than the development cohort (Table 8.3).

All participants in the development study had died, whereas 45% of those in the external validation study were still alive.

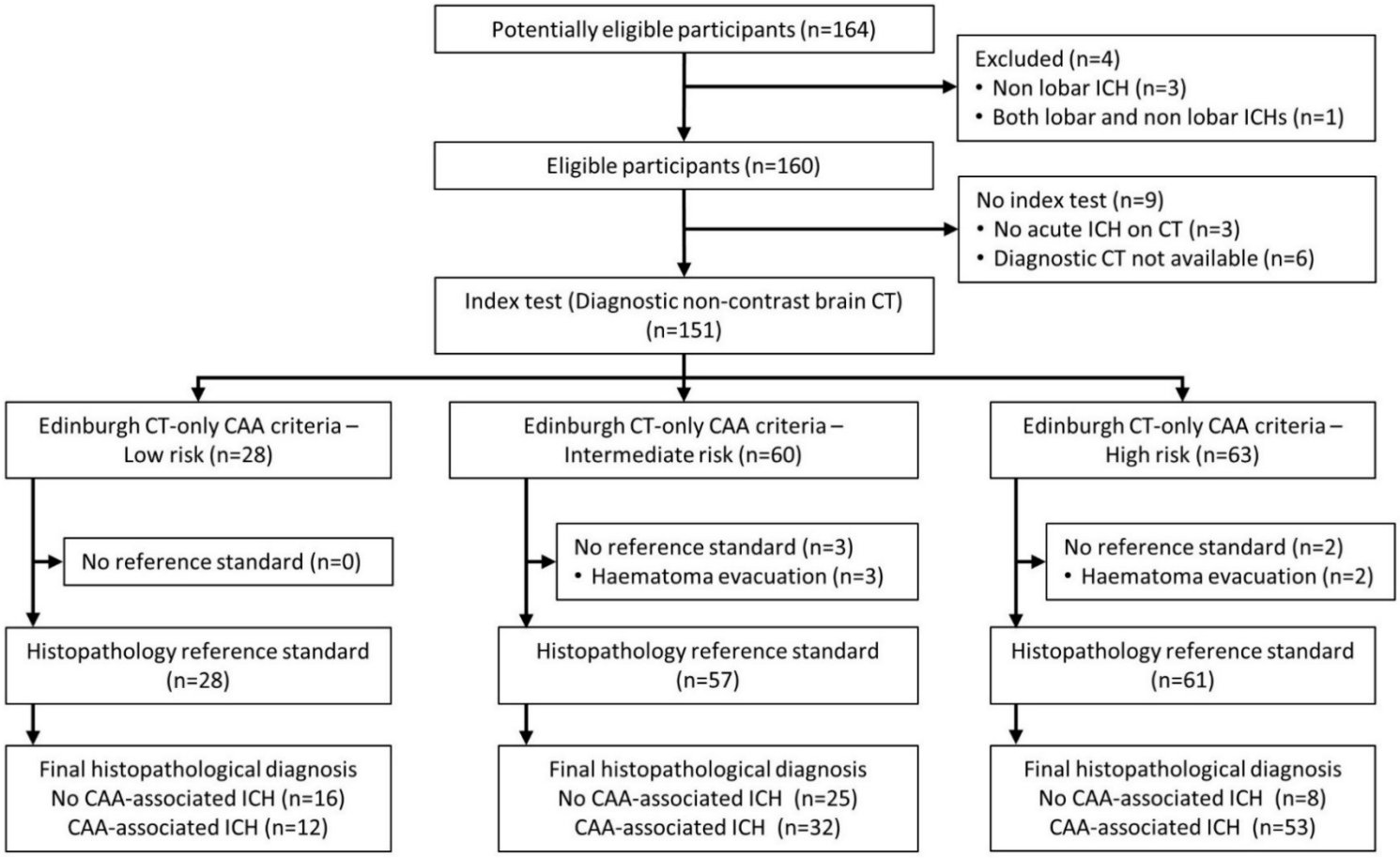
Autopsy was used as the reference standard for 34% of the external validation cohort compared with all participants in the development cohort. Overall, the median time between diagnostic CT and tissue sampling was shorter in the external validation cohort (3 days, IQR 0-13, range 0-2601)

compared with the development cohort (11 days, IQR 6-136, range 1-999). For those undergoing autopsy in the external validation study, the median time between diagnostic CT and tissue sampling was 12 days (IQR 5-516, range 0-2601). Sixty six percent of the external validation cohort were classified as CAA-associated lobar ICH by the reference standard compared with 58% in the development study.

8.4.1.3 Baseline clinical and diagnostic CT brain characteristics in the external validation study of CAA-associated versus non-CAA-associated lobar ICH on histopathology

Ninety seven (66%) participants were classified as CAA-associated lobar ICH and 49 (34%) as non-CAA-associated lobar ICH. Pre-ICH dementia was more common in the CAA-associated lobar ICH group, although this did not reach statistical significance (Table 8.4). Non-CAA-associated lobar ICH participants were more likely to have a pre-ICH history of hypertension. Participants with CAA-associated lobar ICH were more likely to have subarachnoid haemorrhage and finger-like projections as well as more severe white matter lucencies than the non-CAA-associated lobar ICH group (Table 8.5).

Figure 8.1 Flow of participants through the Edinburgh CT only CAA criteria external validation study



CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. Edinburgh CT-only CAA criteria: Low risk = no subarachnoid haemorrhage or finger-like projections; Intermediate risk = subarachnoid haemorrhage or finger-like projections; High risk = subarachnoid haemorrhage and finger-like projections

Table 8.2 Baseline clinical characteristics of the Edinburgh CT criteria development versus external validation cohorts

	Development cohort (n=62)		External validation cohort (n=146)	
Age (years); median (IQR)	83	(78-86)	71	(65-77)
Sex				
Female	39	(63)	91	(62)
Male	23	(37)	55	(38)
Co-morbidities				
Hypertension	42	(68)	90	(62)
Missing	0	(0)	2	(1)
Ischaemic stroke	12	(19)	15	(10)
Missing	0	(0)	2	(1)
Transient ischaemic attack	5	(8)	9	(6)
Missing	0	(0)	6	(4)
Dementia	10	(16)	24	(16)
Missing	0	(0)	23	(16)
Diabetes	6	(10)	18	(12)
Missing	0	(0)	3	(2)
Atrial Fibrillation	19	(31)	23	(16)
Missing	0	(0)	5	(3)
Hyperlipidaemia	8	(13)	42	(29)
Missing	0	(0)	6	(4)
Smoking status				
Current	11	(18)	14	(10)
Ex-smoker	23	(37)	32	(22)
Never	28	(45)	69	(47)
Missing	0	(0)	31	(21)
Medications on admission				
Antiplatelet drug(s)	33	(53)	43	(30)
Missing	0	(0)	3	(2)
Anticoagulant drug(s)	9	(15)	28	(19)
Missing	0	(0)	2	(1)
Admission GCS; median (IQR)	13	(9-14)	12	(8-15)
Missing	0	(0)	37	(25)
APOE genotype	62	(100)	65	(45)
Missing	0	(0)	81	(56)
Time between ICH and diagnostic CT scan (days); median (IQR)	0	(0-1)	0	(0-0)
Tissue source				
Autopsy	62	(100)	50	(34)
Biopsy	0	(0)	96	(66)
Time between diagnostic CT scan and tissue (days); median (IQR)	11	(6-136)	3	(0-13)
CAA-associated lobar ICH	36	(58)	97	(66)
Death	62	(100)	80	(55)

Data are n (%) or median (IQR). * Relates to the largest ICH. APOE = Apolipoprotein. CAA = cerebral amyloid angiopathy .CT = computed tomography. GCS = Glasgow coma scale. ICH = intracerebral haemorrhage.

Table 8.3 Non-contrast diagnostic brain CT characteristics in the Edinburgh CT criteria development versus external validation cohorts

	Development cohort (n=62)		External validation cohort (n=146)	
Multiple acute ICH	9	(15)	16	(11)
ICH location*				
Frontal	29	(47)	79	(54)
Parietal	14	(23)	18	(12)
Temporal	10	(16)	34	(23)
Occipital	9	(15)	14	(10)
Insular	0	(0)	1	(1)
ICH volume (ml)*; median (IQR)	60	(20-118)	56	(33-86)
Strictly lobar ICH	58	(94)	137	(94)
Intraventricular haemorrhage	31	(50)	55	(38)
Subarachnoid haemorrhage	43	(69)	118	(81)
Subdural haemorrhage	12	(19)	37	(25)
Finger-like projections*	14	(23)	61	(42)
Anterior white matter lucencies				
0	10	(16)	47	(32)
1	37	(60)	68	(47)
2	15	(24)	31	(21)
Posterior white matter lucencies				
0	13	(21)	54	(37)
1	9	(15)	23	(16)
2	40	(65)	69	(47)
Central atrophy				
0	19	(31)	60	(41)
1	39	(63)	74	(51)
2	4	(6)	12	(8)
Cortical atrophy				
0	15	(24)	23	(16)
1	33	(53)	105	(72)
2	14	(23)	18	(12)

Data are n (%) or median (IQR). * Relates to the largest ICH. CT = computed tomography. ICH = intracerebral haemorrhage.

Table 8.4 Baseline clinical and non-contrast diagnostic brain CT characteristics in external validation study participants classified as CAA-associated versus non-CAA-associated ICH by the histopathological reference standard

	Non-CAA-associated lobar ICH (n=49)		CAA-associated lobar ICH (n=97)		p value
Age (years); median (IQR)	70	(62-77)	72	(67-79)	0.110
Sex					
Female	28	(57)	63	(65)	0.358
Male	21	(43)	34	(35)	
Co-morbidities					
Hypertension	37	(77)	53	(55)	0.011
Ischaemic stroke	7	(14)	8	(8)	0.275
Transient ischaemic attack^	1	(2)	8	(9)	0.272
Dementia	3	(9)	21	(24)	0.064
Diabetes	7	(15)	11	(12)	0.609
Atrial Fibrillation	7	(15)	16	(17)	0.747
Hyperlipidaemia	15	(33)	27	(29)	0.638
Smoking status^					
Current	7	(18)	7	(9)	0.340
Ex-smoker	11	(29)	21	(27)	
Never	20	(53)	49	(64)	
Medications on admission					
Antiplatelet drug(s)	15	(31)	28	(30)	0.827
Anticoagulant drug(s)	13	(27)	15	(16)	0.101
Admission GCS; median (IQR)	13	(8-15)	12	(8-15)	0.437
Time between ICH and diagnostic CT scan (days); median (IQR)	0	(0-0)	0	(0-0)	
Tissue source					
Autopsy	5	(10)	45	(46)	<0.001
Biopsy	44	(90)	52	(54)	
Time between diagnostic CT scan and tissue (days); median (IQR)	1	(0-7)	4	(1-21)	0.012
Death	14	(29)	66	(68)	<0.001

Data are n (%) or median (IQR). *Relates to the largest ICH. ^Fisher's exact test. CAA = cerebral amyloid angiopathy .CT = computed tomography. GCS = Glasgow coma scale. ICH = intracerebral haemorrhage.

Table 8.5 Non-contrast diagnostic brain CT characteristics in external validation study participants classified as CAA-associated versus non-CAA-associated ICH by the histopathological reference standard

	Non-CAA-associated lobar ICH (n=49)		CAA-associated lobar ICH (n=97)		p value
Multiple acute ICH	5	(10.2)	11	(11.3)	0.836
ICH location*^					
Frontal	26	(53)	53	(55)	
Parietal	4	(8)	14	(14)	
Temporal	14	(29)	20	(21)	0.704
Occipital	5	(10)	9	(9)	
Insular	0	(0)	1	(1)	
ICH volume (ml)*; median (IQR)	50	(20-70)	60	(38-93)	0.082
Strictly lobar ICH	44	(90)	93	(96)	0.149
Intraventricular haemorrhage	18	(37)	37	(38)	0.868
Subarachnoid haemorrhage	33	(67)	85	(88)	0.003
Subdural haemorrhage	13	(27)	24	(25)	0.815
Finger-like projections*	8	(16)	53	(57)	<0.001
Anterior white matter lucencies					
0	25	(51)	22	(23)	
1	20	(41)	48	(50)	0.001
2	4	(8)	27	(28)	
Posterior white matter lucencies					
0	30	(61)	24	(25)	
1	6	(12)	17	(18)	<0.001
2	13	(27)	56	(58)	
Central atrophy^					
0	22	(45)	38	(39)	
1	23	(47)	51	(53)	0.806
2	4	(8)	8	(8)	
Cortical atrophy					
0	5	(10)	18	(19)	
1	35	(71)	70	(72)	0.163
2	9	(18)	9	(9)	
CT SVD score^					
0	28	(57)	34	(35)	
1	16	(33)	48	(50)	0.055
2	5	(10)	11	(11)	
3	0	(0)	4	(4)	

Data are n (%) or median (IQR). *Relates to the largest ICH. ^Fisher's exact test. CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. SVD = small vessel disease.

Participants with a CAA-associated lobar ICH were more likely to have undergone an autopsy and have a longer period between diagnostic CT scanning and tissue sampling than the non-CAA-associated lobar ICH group.

The time between ICH onset and diagnostic non-contrast brain CT ranged from 0 to 7 days. Table 8.6 compares the distribution of brain CT features according to the time between symptom onset and CT scanning. Those scanned on the same day as the symptom onset were more likely to have multiple simultaneous acute ICHs than those scanned on day one onwards. The frequency of subarachnoid haemorrhage and finger-like projections was similar between the two groups.

Table 8.6 Diagnostic non-contrast brain CT features stratified by the timing of the scan relative to ICH symptom onset

	CT day 0 (n=131)		**CT day 1 or later (n=15)**		**p value**
Multiple acute ICH	12	(9)	4	(27)	0.040
ICH location*					
Frontal	69	(53)	10	(67)	
Parietal	16	(12)	2	(13)	
Temporal	32	(24)	2	(13)	0.831
Occipital	13	(10)	1	(7)	
Insular	1	(1)	0	(0)	
Strictly lobar ICH	123	(94)	14	(93)	0.932
ICH volume (ml); median (IQR)	58	(36-89)	38	(9-67)	0.057
Intraventricular haemorrhage	52	(40)	3	(20)	0.136
Subarachnoid haemorrhage	106	(81)	12	(80)	0.932
Subdural haemorrhage	35	(27)	2	(13)	0.259
Finger-like projections	53	(41)	8	(53)	0.338

Data are n (%). CT = computed tomography. ICH = intracerebral haemorrhage.

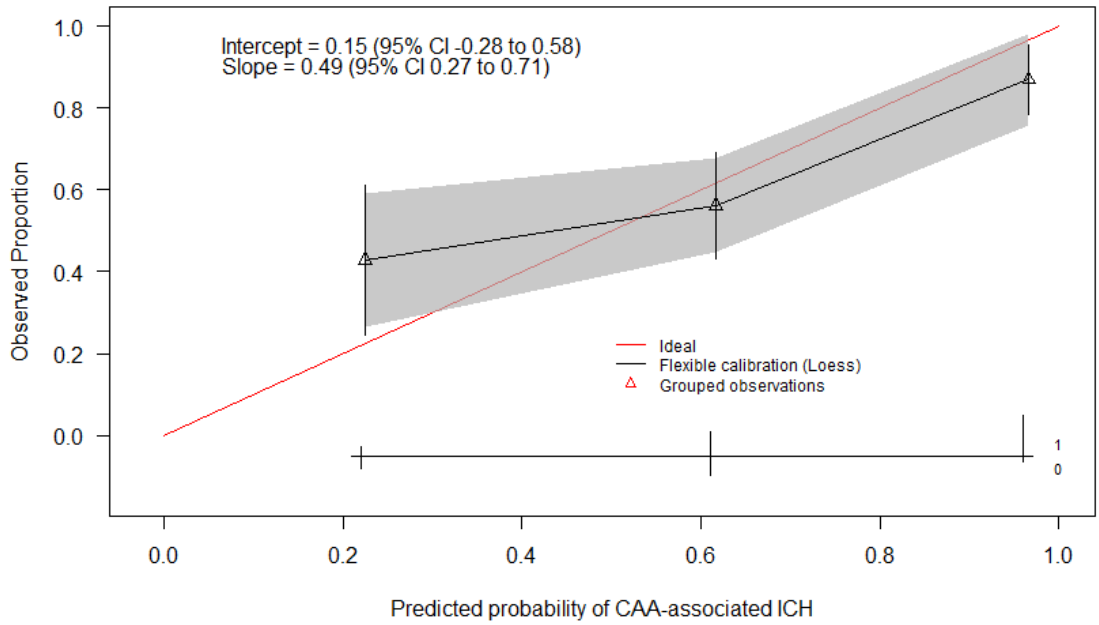
8.4.1.4 Edinburgh CT-only diagnostic model performance

Figure 8.2 shows the model calibration in the external validation cohort. The intercept, which compares the mean predicted risks against observed risks, is 0.15 (95CI -0.59 to 0.27). The calibration slope is 0.49 (95% CI 0.27 to 0.71). The model shows good discrimination (c statistic 0.71, 95% CI 0.63 to 0.79, Figure 8.3).

The net benefit of the model is shown in the decision curves (Figure 8.4). The threshold probability is the level of diagnostic certainty above which a patient or clinician would choose treatment. Equally, it could be used by a researcher to identify patients eligible for inclusion in a randomised controlled trial or for a case-control study. The threshold probability is low in situations where we want to avoid false negatives (e.g. when trying to rule out CAA-associated lobar ICH), and high when false positives are to be avoided (e.g. when trying to rule in CAA-associated lobar ICH). The net benefit is the difference between those expected to benefit (true positives identified using the strategy – expected benefit) and those expected to be harmed (false positives identified using the strategy multiplied by a weighting factor based on the threshold probability – expected harm). The curves which maximise net benefit represent the optimal strategy for the associated threshold probabilities. The solid black line indicates a policy of treating no one (i.e. assume none have CAA-associated lobar ICH), the grey line a policy of treating all (i.e. assume all have CAA-associated lobar ICH).

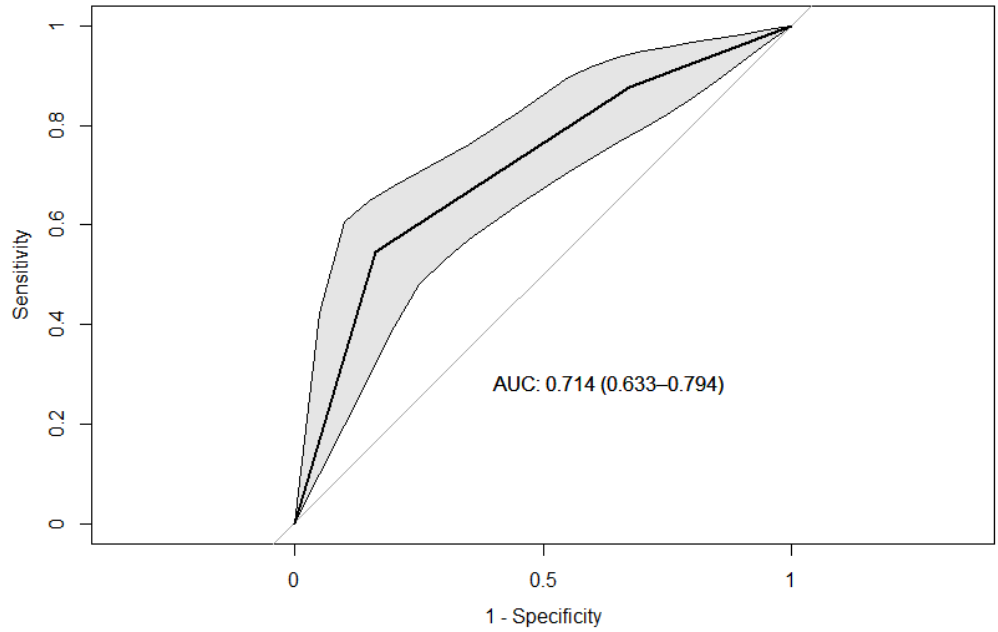
For low threshold probabilities (0-0.4), where harm of unnecessary treatment is limited and false negatives avoided (i.e. useful for ruling out CAA-associated lobar ICH), the line for treat all is above the subarachnoid haemorrhage alone line. Therefore the strategy of treat all maximises net benefit in this situation. For high threshold probabilities (0.6-0.90), where there is harm of overtreatment, and false positives should be avoided, the criteria of subarachnoid haemorrhage AND finger-like projections maximises net benefit (i.e. for ruling in CAA-associated lobar ICH).

Figure 8.2 Calibration plot of predicted probability of CAA-associated lobar ICH using the Edinburgh CT-only prediction model versus the observed frequency of CAA-associated lobar ICH



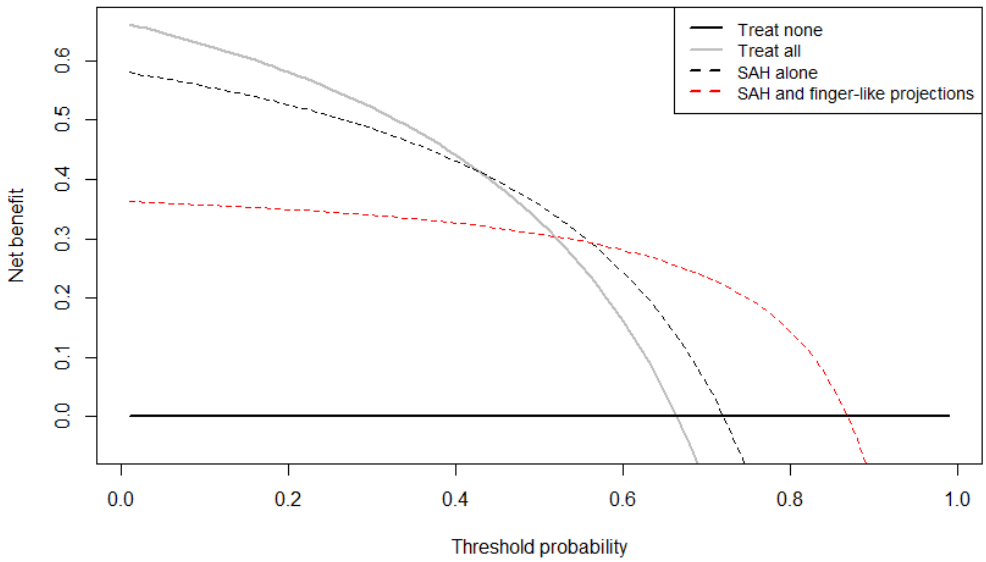
The red line indicates perfect calibration, the model's calibration is shown by the black line. The grey shaded area represents the 95%CI. Triangles represent the three different risk groups produced by the prediction model. Vertical lines at the bottom of the plot represent the distribution of model predicted probabilities stratified by endpoint (CAA-associated ICH above the x-axis, non-CAA-associated ICH below the x-axis). CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage.

Figure 8.3 ROC curve for the predicted probability of CAA-associated lobar ICH using the Edinburgh CT-only prediction model



The AUC is equivalent to the c statistic. The shaded area represents the 95% CI of the AUC based on 2000 bootstrap replicates. The grey line indicates a non-informative AUC of 0.50 for comparison. AUC = area under the curve. CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. ROC = receiver operating characteristic.

Figure 8.4 Decision curves of classifications of CAA-associated lobar ICH using the Edinburgh CT only prediction model



CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. SAH = subarachnoid haemorrhage.

8.4.1.5 Edinburgh CT-only criteria diagnostic accuracy

The cross-tabulation of Edinburgh CT-only diagnostic criteria against the reference standard is shown in Table 8.7. The presence of subarachnoid haemorrhage had a sensitivity of 88% (95% CI 79 to 93) for CAA-associated ICH (Table 8.8), while the combination of subarachnoid haemorrhage and finger-like projections had a specificity of 84% (95% CI 70 to 93).

There were 12 false negative cases for the rule out criteria of subarachnoid haemorrhage (Table 8.7), eight of which had an ICH volume less than 20ml and/or tissue sampling performed more than 1000 days after the ICH resulting in study inclusion. There were eight false positive cases for the rule in criteria of subarachnoid haemorrhage and finger-like projections, all of which were based on brain biopsy.

Table 8.7 Cross-tabulations of the Edinburgh CT-only diagnostic criteria for CAA-associated lobar ICH against the Vonsattel reference standard

Diagnostic criteria (index test)	**Vonsattel grade ≥2 (Reference standard)**		
Subarachnoid haemorrhage	Positive	Negative	Total
Positive	85	33	118
Negative	12	16	28
Total	97	49	146
Diagnostic criteria (index test)	**Vonsattel grade ≥2 (Reference standard)**		
Subarachnoid haemorrhage & finger-like projections	Positive	Negative	Total
Positive	53	8	61
Negative	44	41	85
Total	97	49	146

CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage.

Table 8.8 Diagnostic accuracy statistics for the Edinburgh CT-only diagnostic criteria for CAA-associated lobar ICH in the development and external validation studies

	Development		External validation	
	Subarachnoid haemorrhage	**Subarachnoid haemorrhage & finger-like projections**	**Subarachnoid haemorrhage**	**Subarachnoid haemorrhage & finger-like projections**
Sensitivity	89 (73-96)	39 (24-56)	88 (79-93)	55 (58-74)
Specificity	58 (37-76)	100 (84-100)	33 (20-48)	84 (70-92)
Positive likelihood ratio	2.1 (1.3-3.3)	Inf (NaN-Inf)	1.3 (1.0-1.6)	3.4 (1.7-6.5)
Negative likelihood ratio	0.2 (0.1-0.5)	0.6 (0.5-0.8)	0.4 (0.2-0.7)	0.5 (0.4-0.7)
Positive predictive value	74 (59-86)	100 (73-100)	72 (63-80)	87 (75-94)
Negative predictive value	79 (54-93)	54 (39-68)	57 (37-75)	48 (37-59)

Data are percentage or ratio (95% confidence interval). CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. Inf = infinity. NaN = not a number – calculation cannot be performed because one of the values includes a zero.

Sensitivity analysis – stratifying analyses by tissue source

Table 8.9 and Table 8.10 compare the baseline clinical and non-contrast CT brain characteristics of those undergoing autopsy versus brain biopsy in the external validation study. Those who had a biopsy were significantly younger compared with those undergoing autopsy, with less severe white matter lucencies and less central atrophy. The frequency of dementia, intraventricular haemorrhage and finger-like projections was also lower in the biopsy group, but none of these reached statistical significance. Admission GCS and ICH volume were similar between the groups. The time between diagnostic CT and tissue sampling was significantly longer in those undergoing autopsy (median 12 days (IQR 5-516, range 0-2601) versus 1 day until brain biopsy (IQR 0-4, range 0-2455), $p<0.001$), while the frequency of CAA-associated lobar ICH was higher in the autopsy group.

The Edinburgh CT-only diagnostic model performance in those who had a brain biopsy reference standard and those who had autopsy, are shown in Figure 8.5 to Figure 8.10. Model discrimination was better in the autopsy group compared with the brain biopsy group (c statistic 0.83 versus 0.71 respectively). The diagnostic accuracy of the associated Edinburgh CT-only diagnostic criteria according to the tissue source of the reference standard is shown in Table 8.11 to Table 8.14. The rule out criteria (subarachnoid haemorrhage) have better sensitivity in the brain biopsy group, whereas the rule in criteria (subarachnoid haemorrhage and finger-like projections) were more specific in the autopsy group.

Table 8.9 Baseline clinical characteristics in external validation study participants who underwent autopsy versus brain biopsy reference standard

	Autopsy (n=50)		Brain biopsy (n=96)		p value
Age (years); median (IQR)	75	(70-83)	70	(64-76)	<0.001
Sex					
Female	29	(58)	62	(65)	0.436
Male	21	(42)	34	(35)	
Co-morbidities					
Hypertension	32	(64)	58	(62)	0.786
Ischaemic stroke	4	(8)	11	(12)	0.525
Transient ischaemic attack^	4	(8)	5	(5)	0.493
Dementia	13	(27)	11	(15)	0.090
Diabetes	5	(10)	13	(14)	0.494
Atrial Fibrillation	8	(16)	15	(16)	0.997
Hyperlipidaemia	14	(29)	28	(31)	0.787
Smoking status					
Current	3	(7)	11	(15)	0.416
Ex-smoker	13	(30)	19	(26)	
Never	27	(63)	42	(58)	
Medications on admission					
Antiplatelet drug(s)	12	(25)	31	(33)	0.293
Anticoagulant drug(s)	9	(18)	19	(20)	0.815
Admission Glasgow Coma Scale Median (IQR)	11	(8-14)	13	(8-15)	0.345
APOE genotype	26	(52)	39	(41)	0.189
APOE ε2 possession	10	(39)	11	(28)	0.386
APOE ε4 possession	13	(50)	15	(39)	0.357
CAA-associated lobar ICH	45	(90)	52	(54)	<0.001
Time between diagnostic CT scan and tissue (days); Median (IQR)	12	(5-516)	1	(0-4)	<0.001
Dead	50	(100)	30	(31)	<0.001

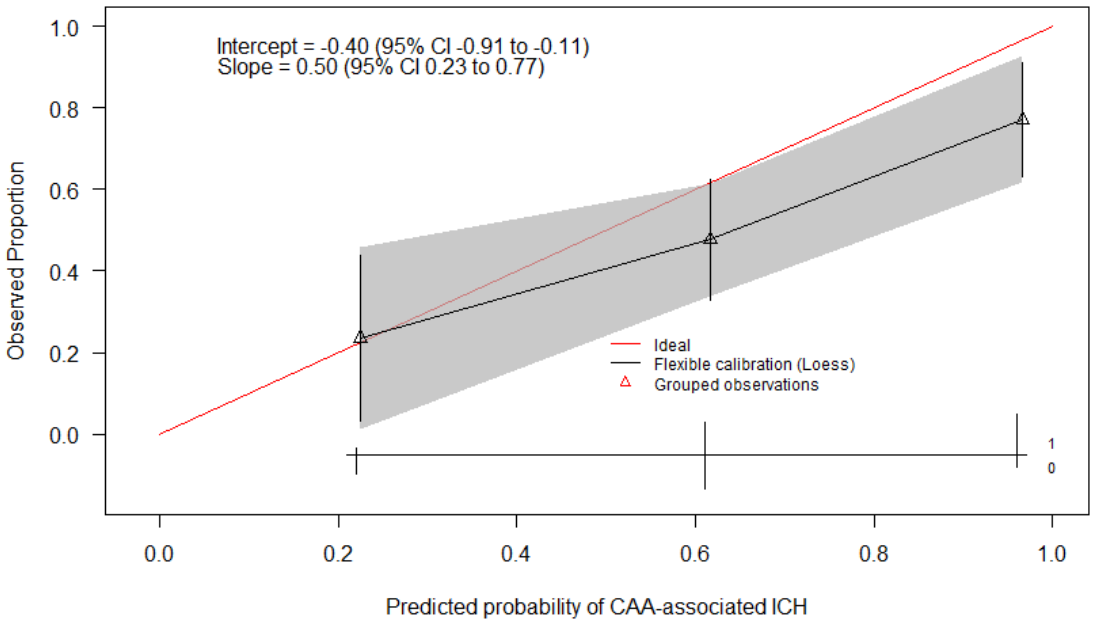
Data are n (%) or median (IQR). * Relates to the largest ICH. ^ Fisher's exact test. APOE = Apolipoprotein. CAA = cerebral amyloid angiopathy .CT = computed tomography. GCS = Glasgow coma scale. ICH = intracerebral haemorrhage.

Table 8.10 Non-contrast diagnostic brain CT characteristics in external validation study participants who underwent autopsy versus brain biopsy reference standard

	Autopsy (n=50)		Brain biopsy (n=96)		p value
Multiple acute ICH	5	(10)	11	(12)	0.789
ICH location*^					
Frontal	26	(52)	53	(55)	
Parietal	4	(8)	14	(15)	
Temporal	10	(20)	24	(25)	0.056
Occipital	9	(18)	5	(5)	
Insular	1	(2)	0	(0)	
Strictly lobar ICH	46	(92)	91	(95)	0.506
ICH volume Median (IQR)	55	(21-79)	57	(39-91)	0.321
Intraventricular haemorrhage	22	(44)	33	(34)	0.255
Subarachnoid haemorrhage	39	(78)	79	(82)	0.532
Subdural haemorrhage	11	(22)	26	(27)	0.503
Finger-like projections	26	(52)	35	(37)	0.071
Anterior white matter lucencies					
0	9	(18)	38	(40)	
1	22	(44)	46	(48)	0.001
2	19	(38)	12	(13)	
Posterior white matter lucencies					
0	11	(22)	43	(45)	
1	7	(14)	16	(17)	0.010
2	32	(64)	37	(39)	
Central atrophy^					
0	11	(22)	49	(51)	
1	32	(64)	42	(44)	0.002
2	7	(14)	5	(5)	
Cortical atrophy					
0	7	(14)	16	(17)	
1	37	(74)	68	(71)	0.904
2	6	(12)	12	(13)	
CT SVD score^					
0	14	(28)	48	(50)	
1	24	(48)	40	(42)	
2	9	(18)	7	(7)	0.012
3	3	(6)	1	(1)	

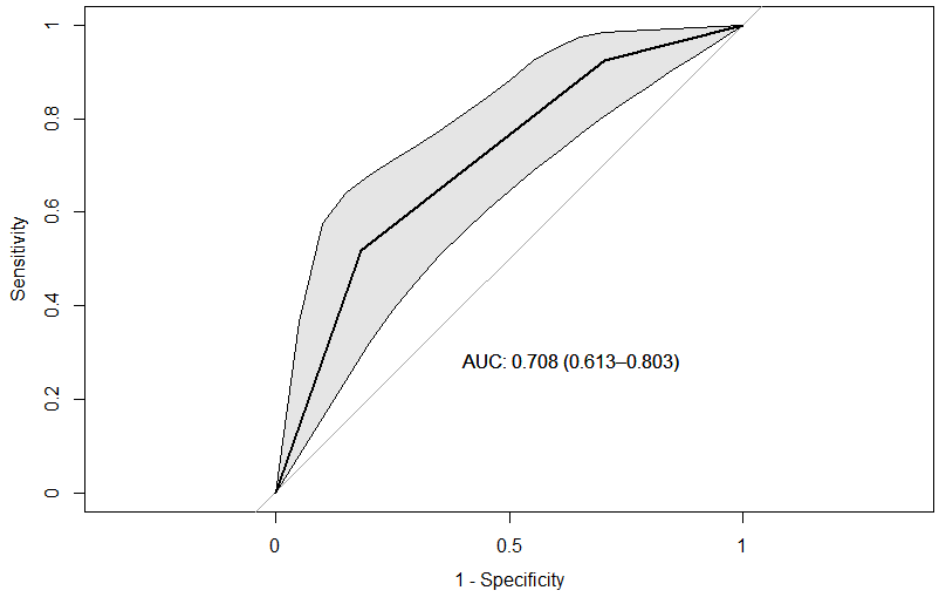
Data are n (%) or median (IQR). * Relates to the largest ICH. ^ Fisher's exact test. CT = computed tomography. ICH = intracerebral haemorrhage. SVD = small vessel disease.

Figure 8.5 Calibration plot of predicted probability of CAA-associated lobar ICH using the Edinburgh CT-only prediction model versus the observed frequency of CAA-associated lobar ICH for those who had a brain biopsy



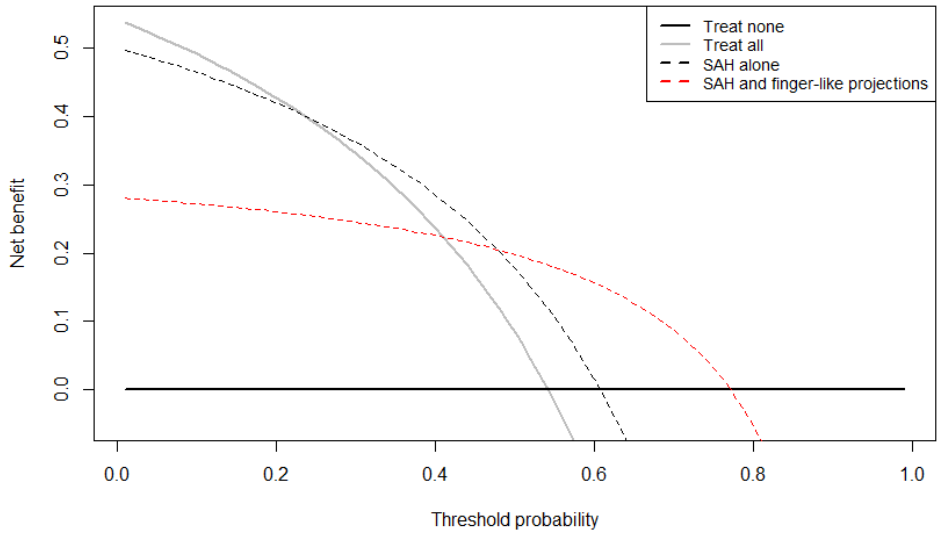
The red line indicates perfect calibration, the model's calibration is shown by the black line. The grey shaded area represents the 95%CI. Triangles represent the three different risk groups produced by the prediction model. Vertical lines at the bottom of the plot represent the distribution of model predicted probabilities stratified by endpoint (CAA-associated ICH above the x-axis, non-CAA-associated ICH below the x-axis). CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage.

Figure 8.6 ROC curve for the predicted probability of CAA-associated lobar ICH using the Edinburgh CT-only prediction model for those who had a brain biopsy



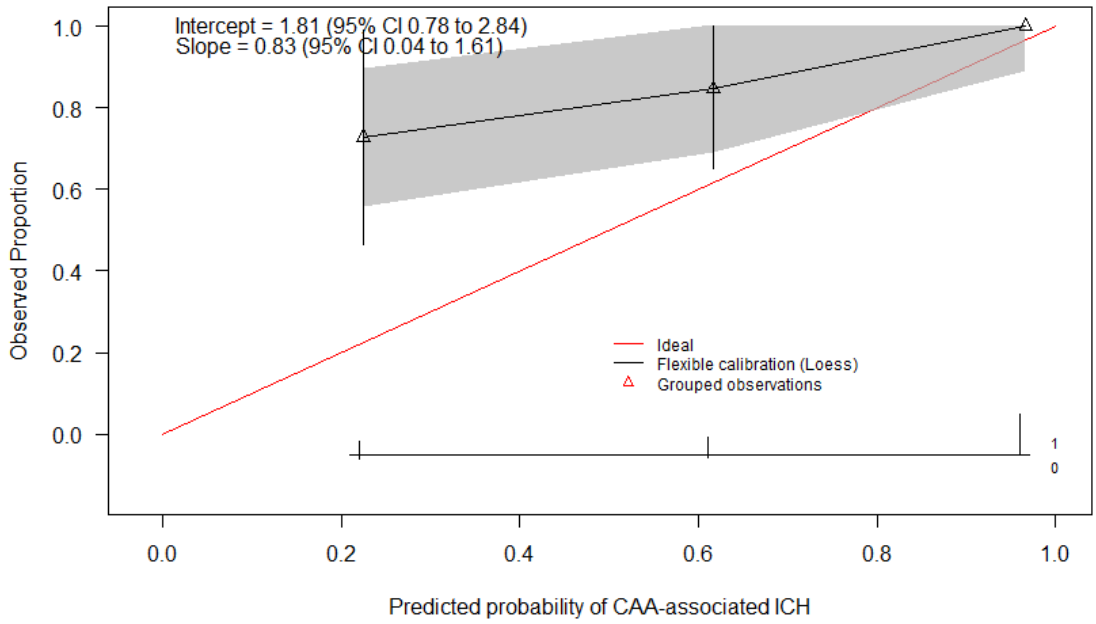
The AUC is equivalent to the c statistic. The shaded area represents the 95% CI of the AUC based on 2000 bootstrap replicates. The grey line indicates a non-informative AUC of 0.50. AUC = area under the curve. CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. ROC = receiver operating characteristic.

Figure 8.7 Decision curves of predictions and classifications of CAA-associated lobar ICH using the Edinburgh CT only prediction model in participants who had a brain biopsy reference standard



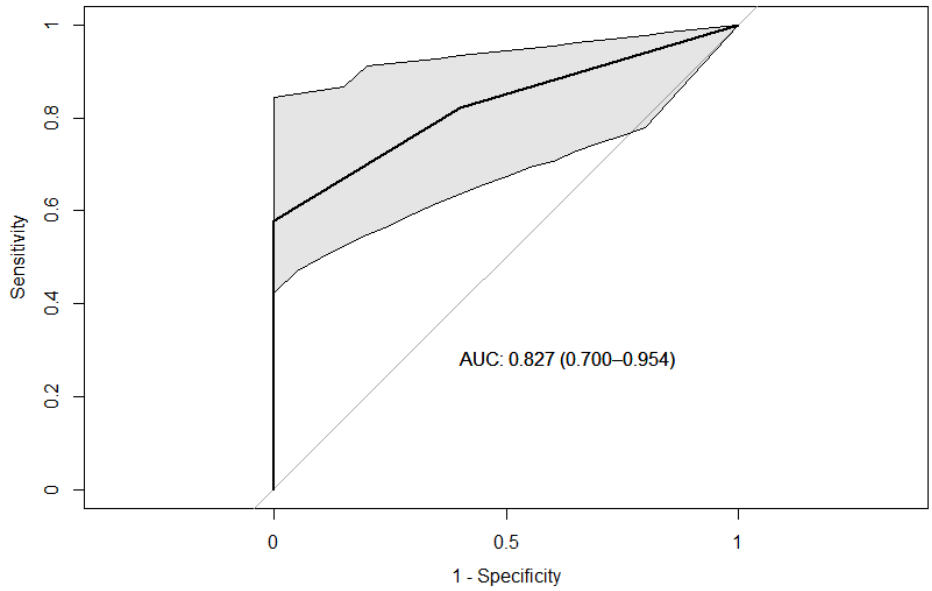
CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. SAH = subarachnoid haemorrhage.

Figure 8.8 Calibration plot of predicted probability of CAA-associated lobar ICH using the Edinburgh CT-only prediction model versus the observed frequency of CAA-associated lobar ICH for those who had an autopsy



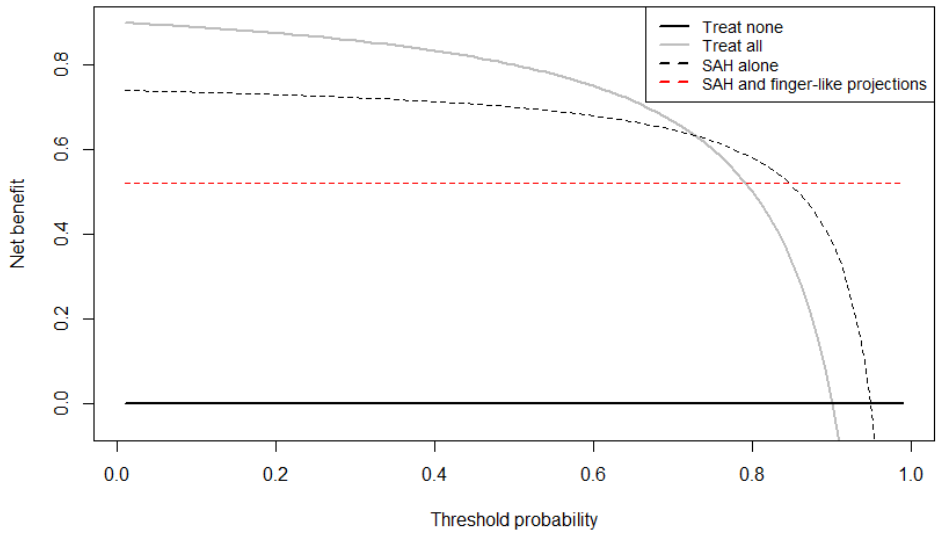
The red line indicates perfect calibration, the model's calibration is shown by the black line. The grey shaded area represents the 95%CI. Triangles represent the three different risk groups produced by the prediction model. Vertical lines at the bottom of the plot represent the distribution of model predicted probabilities stratified by endpoint (CAA-associated ICH above the x-axis, non-CAA-associated ICH below the x-axis). CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage.

Figure 8.9 ROC curve for the predicted probability of CAA-associated lobar ICH using the Edinburgh CT-only prediction model for those who had an autopsy



The AUC is equivalent to the c statistic. The shaded area represents the 95% CI of the AUC based on 2000 bootstrap replicates. The grey line indicates a non-informative AUC of 0.50. AUC = area under the curve. CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. ROC = receiver operating characteristic.

Figure 8.10 Decision curves of predictions and classifications of CAA-associated lobar ICH using the Edinburgh CT only prediction model in participants who had an autopsy reference standard



CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. SAH = subarachnoid haemorrhage

Table 8.11 Cross-tabulations of the Edinburgh CT-only diagnostic criteria for CAA-associated lobar ICH against the Vonsattel reference standard in those who underwent brain biopsy

Diagnostic criteria (index test)	**Vonsattel grade ≥2 (Reference standard)**		
Subarachnoid haemorrhage	Positive	Negative	Total
Positive	48	31	79
Negative	4	13	17
Total	52	44	96
Diagnostic criteria (index test)	**Vonsattel grade ≥2 (Reference standard)**		
Subarachnoid haemorrhage & finger-like projections	Positive	Negative	Total
Positive	27	8	35
Negative	25	36	61
Total	52	44	96

CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage.

Table 8.12 Diagnostic accuracy statistics for the Edinburgh CT-only diagnostic criteria for CAA-associated lobar ICH in those who underwent brain biopsy

	Edinburgh CT-only diagnostic criteria	
	Subarachnoid haemorrhage	**Subarachnoid haemorrhage & finger-like projections**
Sensitivity	92 (81-98)	53 (38-66)
Specificity	30 (17-45)	82 (67-91)
Positive likelihood ratio	1.3 (1.1-1.6)	2.9 (1.5-5.6)
Negative likelihood ratio	0.3 (0.1-0.7)	0.6 (0.4-0.8)
Positive predictive value	61 (49-71)	77 (59-89)
Negative predictive value	77 (50-92)	59 (46-71)

Data are percentage or ratio (95% confidence interval). CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage.

Table 8.13 Cross-tabulations of the Edinburgh CT-only diagnostic criteria for CAA-associated lobar ICH against the Vonsattel reference standard in those who underwent autopsy

Diagnostic criteria (index test)	**Vonsattel grade ≥2 (Reference standard)**		
Subarachnoid haemorrhage	Positive	Negative	Total
Positive	37	2	39
Negative	8	3	11
Total	45	5	50
Diagnostic criteria (index test)	**Vonsattel grade ≥2 (Reference standard)**		
Subarachnoid haemorrhage & finger-like projections	Positive	Negative	Total
Positive	26	0	26
Negative	19	5	24
Total	45	5	50

CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage.

Table 8.14 Diagnostic accuracy statistics for the Edinburgh CT-only diagnostic criteria for CAA-associated lobar ICH in those who underwent autopsy

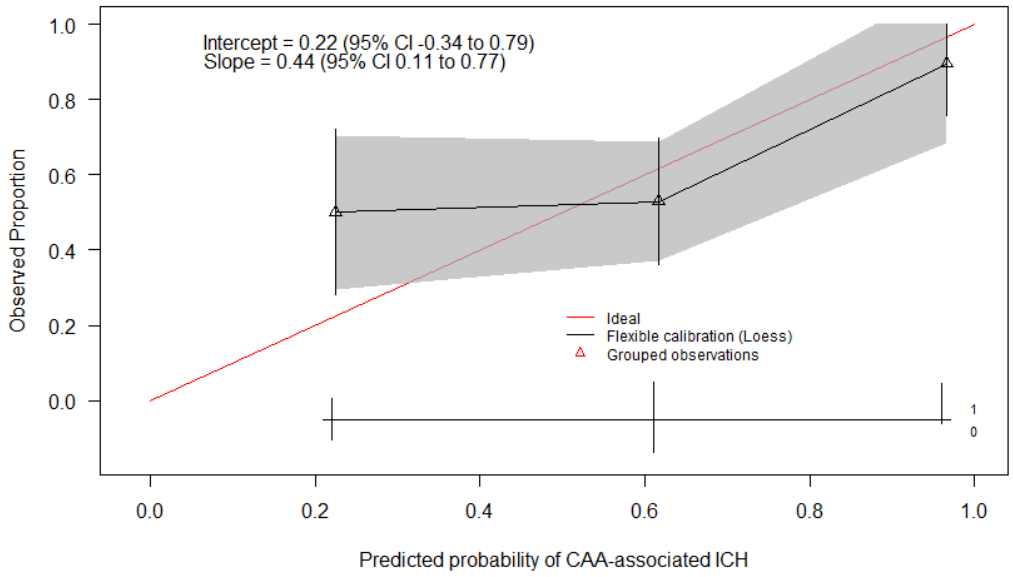
	Edinburgh CT-only diagnostic criteria			
	Subarachnoid haemorrhage		**Subarachnoid haemorrhage & finger-like projections**	
Sensitivity	82	(67-92)	58	(42-72)
Specificity	60	(17-93)	100	(46-100)
Positive likelihood ratio	2.1	(0.7-6.1)	Inf	(NaN-Inf)
Negative likelihood ratio	0.3	(0.1-0.7)	0.4	(0.3-0.6)
Positive predictive value	95	(81-99)	100	(84-100)
Negative predictive value	27	(7-61)	21	(8-43)

Data are percentage or ratio (95% confidence interval). CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. Inf = infinity. NaN = not a number – calculation cannot be performed because one of the values includes a zero.

8.4.1.6 Sensitivity analysis – stratifying analyses by ICH volume

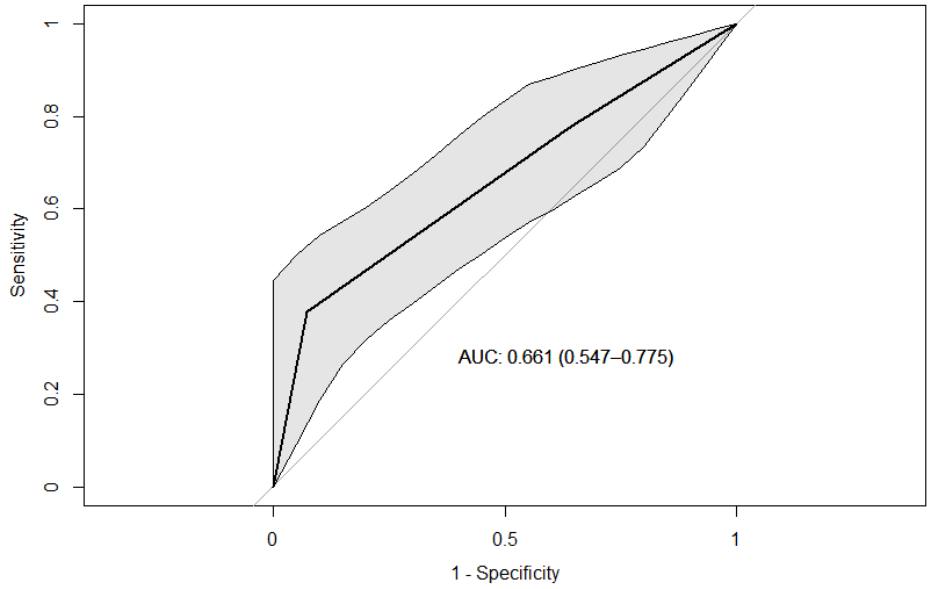
Figure 8.11 to Figure 8.16 show the Edinburgh CT-only diagnostic model performance stratified by the median ICH volume. In the group with smaller ICHs, the model underestimated the frequency of CAA-associated lobar ICH for the low predicted model probabilities and showed modest discrimination (c statistic 0.66). In the group with larger ICHs, the model showed good calibration across all model predicted probabilities and good discrimination (c statistic 0.73). The diagnostic accuracy of the associated Edinburgh CT-only diagnostic criteria is shown in Table 8.15 to Table 8.18. The sensitivity of the rule out criteria (subarachnoid haemorrhage) was best in the larger ICH group, whereas the rule in criteria (subarachnoid haemorrhage and finger-like projections) were more specific in the smaller ICH group.

Figure 8.11 Calibration plot of predicted probability of CAA-associated lobar ICH using the Edinburgh CT-only prediction model versus the observed frequency of CAA-associated lobar ICH for those with an ICH volume below 56 ml



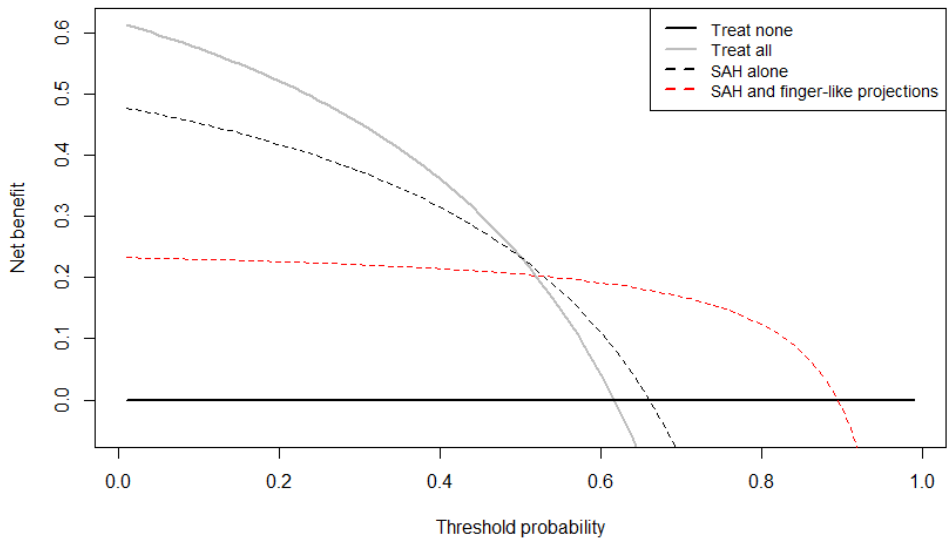
The red line indicates perfect calibration, the model's calibration is shown by the black line. The grey shaded area represents the 95%CI. Triangles represent the three different risk groups produced by the prediction model. Vertical lines at the bottom of the plot represent the distribution of model predicted probabilities stratified by endpoint (CAA-associated ICH above the x-axis, non-CAA-associated ICH below the x-axis). CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage.

Figure 8.12 ROC curve for the predicted probability of CAA-associated lobar ICH using the Edinburgh CT-only prediction model for those with an ICH volume below 56 ml



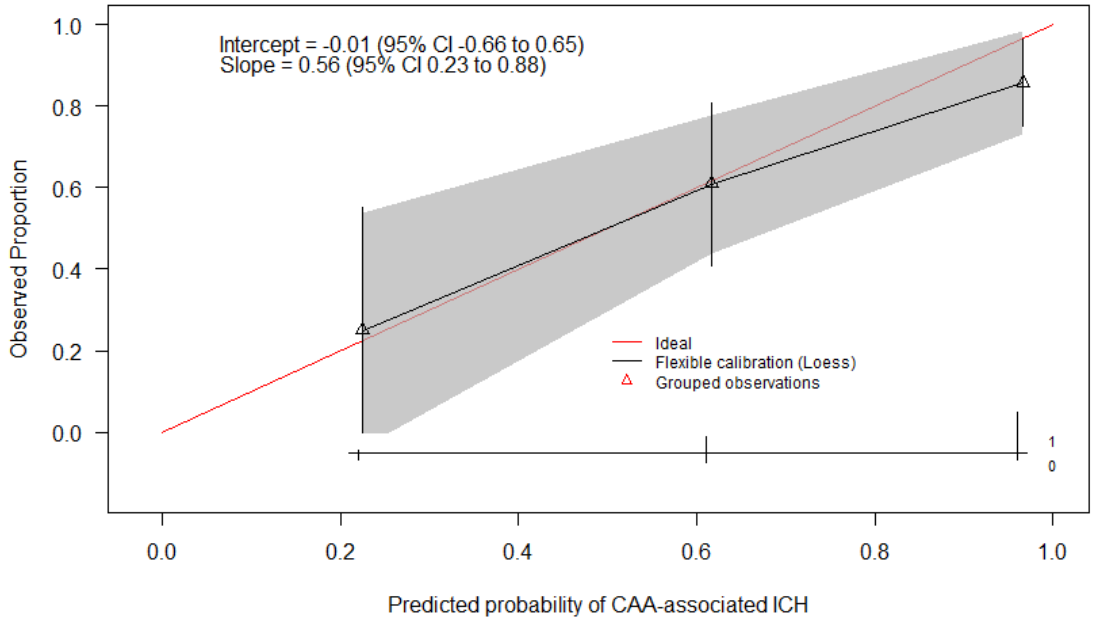
The AUC is equivalent to the c statistic. The shaded area represents the 95% CI of the AUC based on 2000 bootstrap replicates. The grey line indicates a non-informative AUC of 0.50. AUC = area under the curve. CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. ROC receiver operating characteristic.

Figure 8.13 Decision curves of predictions and classifications of CAA-associated lobar ICH using the Edinburgh CT only prediction model in participants patients who had an ICH volume below 56 ml



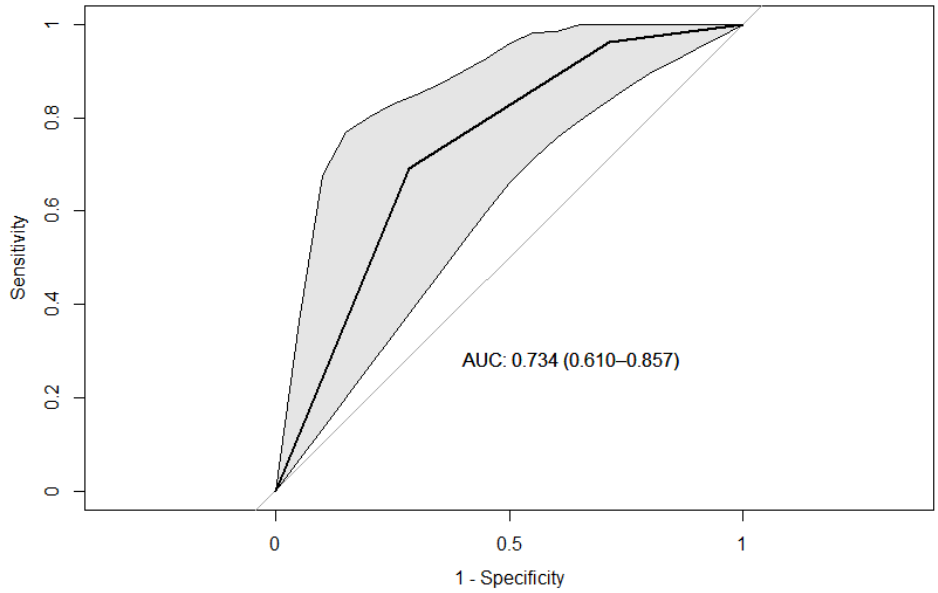
CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. SAH = subarachnoid haemorrhage

Figure 8.14 Calibration plot of predicted probability of CAA-associated lobar ICH using the Edinburgh CT-only prediction model versus the observed frequency of CAA-associated lobar ICH for those with an ICH volume above 56 ml



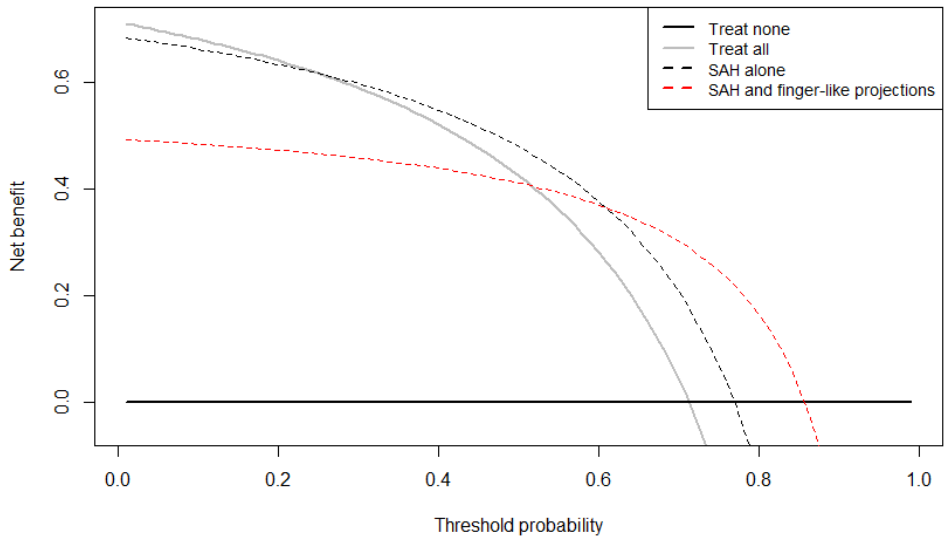
The red line indicates perfect calibration, the model's calibration is shown by the black line. The grey shaded area represents the 95%CI. Triangles represent the three different risk groups produced by the prediction model. Vertical lines at the bottom of the plot represent the distribution of model predicted probabilities stratified by endpoint (CAA-associated ICH above the x-axis, non-CAA-associated ICH below the x-axis). CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage.

Figure 8.15 ROC curve for the predicted probability of CAA-associated lobar ICH using the Edinburgh CT-only prediction model for those with an ICH volume above 56 ml



The AUC is equivalent to the c statistic. The shaded area represents the 95% CI of the AUC based on 2000 bootstrap replicates. The grey line indicates a non-informative AUC of 0.50. AUC = area under the curve. CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. ROC = receiver operating characteristic.

Figure 8.16 Decision curves of predictions and classifications of CAA-associated lobar ICH using the Edinburgh CT only prediction model in participants who had an ICH volume above 56 ml



CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. SAH = subarachnoid haemorrhage.

Table 8.15 Cross-tabulations of the Edinburgh CT-only diagnostic criteria for CAA-associated lobar ICH against the Vonsattel reference standard in those with ICH volume less than 56 ml

Diagnostic criteria (index test)	**Vonsattel grade ≥2 (Reference standard)**		
Subarachnoid haemorrhage	Positive	Negative	Total
Positive	35	18	53
Negative	10	10	20
Total	45	28	73
Diagnostic criteria (index test)	**Vonsattel grade ≥2 (Reference standard)**		
Subarachnoid haemorrhage & finger-like projections	Positive	Negative	Total
Positive	17	2	19
Negative	28	26	54
Total	45	28	73

CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage.

Table 8.16 Diagnostic accuracy statistics for the Edinburgh CT-only diagnostic criteria for CAA-associated lobar ICH in those with ICH volume less than 56 ml

	Edinburgh CT-only diagnostic criteria	
	Subarachnoid haemorrhage	**Subarachnoid haemorrhage & finger-like projections**
Sensitivity	78 (63-89)	38 (24-54)
Specificity	36 (19-56)	93 (75-99)
Positive likelihood ratio	1.2 (0.9-1.7)	5.3 (1.3-21)
Negative likelihood ratio	0.6 (0.3-1.2)	0.7 (0.5-0.9)
Positive predictive value	66 (52-78)	90 (66-98)
Negative predictive value	50 (28-72)	48 (35-62)

Data are percentage or ratio (95% confidence interval). CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage.

Table 8.17 Cross-tabulations of the Edinburgh CT-only diagnostic criteria for CAA-associated lobar ICH against the Vonsattel reference standard in those with ICH volume more than 56 ml

Diagnostic criteria (index test)	**Vonsattel grade ≥2 (Reference standard)**		
Subarachnoid haemorrhage	Positive	Negative	Total
Positive	49	15	64
Negative	2	6	8
Total	51	21	72
Diagnostic criteria (index test)	**Vonsattel grade ≥2 (Reference standard)**		
Subarachnoid haemorrhage & finger-like projections	Positive	Negative	Total
Positive	36	6	42
Negative	15	15	30
Total	51	21	72

CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage.

Table 8.18 Diagnostic accuracy statistics for the Edinburgh CT-only diagnostic criteria for CAA-associated lobar ICH in those with ICH volume more than 56 ml

	Edinburgh CT-only diagnostic criteria	
	Subarachnoid haemorrhage	**Subarachnoid haemorrhage & finger-like projections**
Sensitivity	96 (86-99)	69 (55-80)
Specificity	29 (12-52)	71 (48-88)
Positive likelihood ratio	1.4 (1.0-1.8)	2.4 (1.2-4.9)
Negative likelihood ratio	0.1 (0.0-0.7)	0.4 (0.3-0.7)
Positive predictive value	77 (65-86)	86 (71-94)
Negative predictive value	75 (36-96)	58 (31-67)

Data are percentage or ratio (95% confidence interval). CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage.

8.4.1.7 Sensitivity analysis – restricting analyses to those where tissue sampling occurred within 100 days of the diagnostic CT scan

There are difficulties relating findings on the diagnostic brain CT to the severity of CAA on a delayed brain tissue sample due to the potential interval development and progression of CAA with time. This could cause a false positive reference standard result. So, I assessed the diagnostic accuracy of the CT-only criteria in the 125 participants who had tissue sampling within 100 days of the diagnostic CT (Table 8.19 and Table 8.20). The sensitivity of the rule out criteria (subarachnoid haemorrhage) was higher in this subgroup compared with the entire cohort (94% versus 88% respectively), while the specificity was similar (83% versus 84%).

Table 8.19 Cross-tabulations of the Edinburgh CT-only diagnostic criteria for CAA-associated lobar ICH against the Vonsattel reference standard in those where pathology tissue was acquired within 100 days of the CT scan

Diagnostic criteria (index test)	**Vonsattel grade ≥2 (Reference standard)**		
Subarachnoid haemorrhage	Positive	Negative	Total
Positive	73	32	105
Negative	5	15	20
Total	78	47	125
Diagnostic criteria (index test)	**Vonsattel grade ≥2 (Reference standard)**		
Subarachnoid haemorrhage & finger-like projections	Positive	Negative	Total
Positive	45	8	53
Negative	33	39	72
Total	78	47	125

CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage.

Table 8.20 Diagnostic accuracy statistics for the Edinburgh CT-only diagnostic criteria for CAA-associated lobar ICH in those where pathology tissue was acquired within 100 days of the CT scan

	Edinburgh CT-only diagnostic criteria	
	Subarachnoid haemorrhage	**Subarachnoid haemorrhage & finger-like projections**
Sensitivity	94 (85-98)	58 (46-69)
Specificity	32 (20-47)	83 (69-92)
Positive likelihood ratio	1.4 (1.1-1.7)	3.4 (1.8-6.6)
Negative likelihood ratio	0.2 (0.1-0.5)	0.5 (0.4-0.7)
Positive predictive value	70 (60-78)	85 (72-93)
Negative predictive value	75 (51-90)	54 (42-66)

Data are percentage or ratio (95% confidence interval). CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage.

8.4.2 External validation of the Edinburgh CT and APOE diagnostic model and criteria

8.4.2.1 Flow of participants

Only 65 (45%) of the 146 participants included in the external validation of the CT-only diagnostic model and criteria had APOE genotyping available (Figure 8.17).

8.4.2.2 Comparison of participants with APOE genotyping in the external validation study versus those without APOE genotyping

Participants with APOE genotyping had similar demographic and baseline clinical and non-contrast CT brain characteristics to those without APOE genotyping. Histopathologically defined CAA-associated ICH was more frequent in those with available APOE genotype (75% versus 59%, p=0.040) (Table 8.21 and Table 8.22).

8.4.2.3 Comparison of participants included in the external validation study with those in the development study

Participants in the CT and APOE external validation cohort tended to be younger than those in the development cohort (Table 8.23), with a lower

frequency of previous ischaemic stroke and of atrial fibrillation. Hyperlipidaemia was more common in the external validation cohort. The frequency of other comorbidities was similar between the external validation and development cohorts. Fewer participants in the external validation cohort were taking an antiplatelet drug at the time of ICH. Admission GCS and ICH volume were similar between the external validation and development cohorts (Table 8.24).

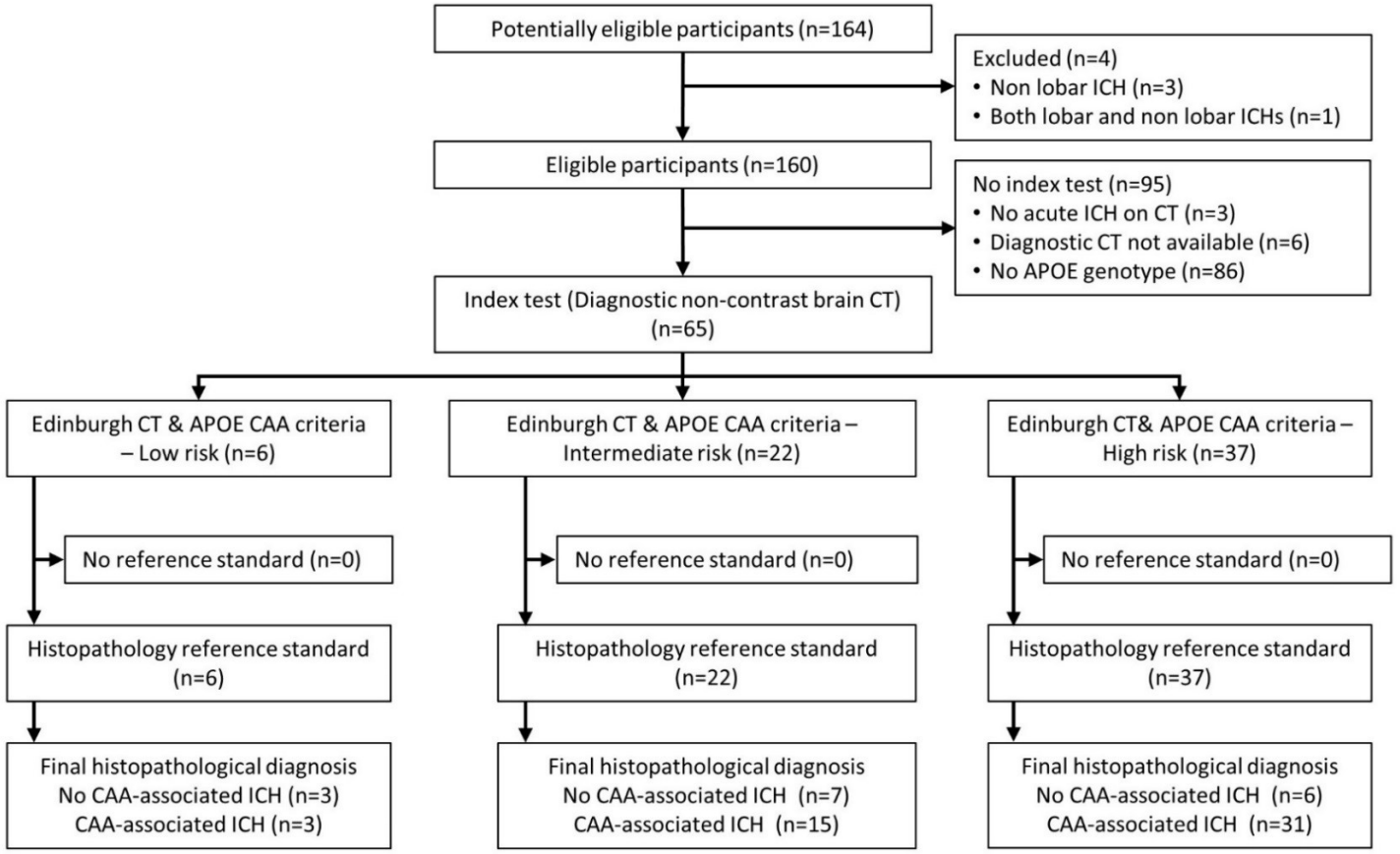
Subarachnoid haemorrhage and finger-like projections were more frequent in the external validation cohort than in the development cohort (Table 8.24).

An autopsy was used as the reference standard for only 40% of the external validation cohort compared with all participants in the development cohort. The median time from diagnostic CT until tissue sampling was two days (IQR 1-16, range 0-2601) in the external validation cohort compared with 11 days (IQR 6-136, range 1-999) in the development cohort. 75% of the external validation cohort were classified as CAA-associated lobar ICH by the reference standard compared with 58% in the development study.

8.4.2.4 Baseline clinical and diagnostic CT brain characteristics in the external validation study of CAA-associated versus non-CAA-associated lobar ICH on histopathology

Forty nine (75%) participants were classified as CAA-associated lobar ICH and 16 (25%) as non-CAA-associated lobar ICH. Pre-ICH dementia, strictly lobar ICH, subarachnoid haemorrhage and finger-like projections were more common in the CAA-associated lobar ICH group, although these did not reach statistical significance (Table 8.25 and Table 8.26).

Figure 8.17 Flow of participants through the Edinburgh CT and APOE CAA criteria external validation study



APOE = apolipoprotein E. CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage.
Edinburgh CT & APOE CAA criteria: Low risk = no subarachnoid haemorrhage, APOE ε4 or finger-like projections; Intermediate risk = either subarachnoid haemorrhage or APOE ε4; High risk = subarachnoid haemorrhage and (APOE ε4 and/or finger-like projections)

Table 8.21 Baseline clinical and non-contrast diagnostic brain CT characteristics in those with and without APOE genotyping

	No APOE genotyping (n=81)		APOE genotyping (n=65)		p value
Age (years); median (IQR)	72	(67-78)	70	(65-77)	0.388
Sex					
Female	52	(64)	39	(60)	0.603
Male	29	(36)	26	(40)	
Co-morbidities					
Hypertension	53	(67)	37	(57)	0.210
Ischaemic stroke	8	(10)	7	(11)	0.900
Transient ischaemic attack^	5	(7)	4	(6)	1.000
Dementia	12	(21)	12	(19)	0.756
Diabetes	12	(15)	6	(9)	0.269
Atrial Fibrillation	14	(18)	9	(14)	0.510
Hyperlipidaemia	24	(32)	18	(28)	0.579
Smoking status					
Current	9	(14)	5	(10)	
Ex-smoker	13	(20)	19	(37)	0.128
Never	42	(66)	27	(53)	
Medications on admission					
Antiplatelet drug(s)	27	(35)	16	(25)	0.194
Anticoagulant drug(s)	18	(23)	10	(15)	0.264
Admission GCS; median (IQR)	13	(8-15)	11	(8-14)	0.649
CAA-associated lobar ICH	48	(59)	49	(75)	0.040
Time between diagnostic CT scan and tissue (days); median (IQR)	3	(0-10)	1	(1-16)	0.613
Dead	36	(44)	44	(68)	0.005

Data are n (%) or median (IQR). * Relates to the largest ICH. ^ Fisher's exact test. APOE = Apolipoprotein. CAA = cerebral amyloid angiopathy .CT = computed tomography. GCS = Glasgow coma scale. ICH = intracerebral haemorrhage.

Table 8.22 Non-contrast diagnostic brain CT characteristics between those with and without APOE genotyping

	No APOE genotyping (n=81)		APOE genotyping (n=65)		p value
Multiple acute ICH	9	(11)	7	(11)	0.948
ICH location*^					
Frontal	43	(53)	36	(55)	
Parietal	12	(15)	6	(9)	
Temporal	16	(20)	18	(28)	0.565
Occipital	9	(11)	5	(8)	
Insular	1	(1)	0	(0)	
Strictly lobar ICH	77	(95)	60	(92)	0.492
ICH volume (ml); median (IQR)	58	(30-95)	55	(37-81)	0.615
Intraventricular haemorrhage	30	(37)	25	(39)	0.860
Subarachnoid haemorrhage	65	(80)	53	(82)	0.844
Subdural haemorrhage	22	(27)	15	(23)	0.573
Finger-like projections*	36	(44)	25	(39)	0.466
Anterior white matter lucencies					
0	32	(40)	15	(23)	
1	37	(46)	31	(48)	0.037
2	12	(15)	19	(29)	
Posterior white matter lucencies					
0	34	(42)	20	(31)	
1	11	(14)	12	(19)	0.354
2	36	(44)	33	(51)	
Central atrophy					
0	34	(42)	26	(40)	
1	39	(48)	35	(54)	0.646
2	8	(10)	4	(6)	
Cortical atrophy					
0	13	(16)	10	(15)	
1	53	(65)	52	(80)	0.035
2	15	(19)	3	(5)	
CT SVD score^					
0	37	(46)	25	(39)	
1	29	(36)	35	(54)	0.102
2	12	(15)	4	(6)	
3	3	(4)	1	(2)	

Data are n (%) or median (IQR). * Relates to the largest ICH. ^ Fisher's exact test. APOE = apolipoprotein E. CT = computed tomography. ICH = intracerebral haemorrhage. SVD = small vessel disease.

Table 8.23 Baseline clinical and non-contrast diagnostic brain CT characteristics in the Edinburgh CT and APOE criteria development versus external validation cohorts

	Development cohort (n=62)		**External validation cohort (n=65)**	
Age (years); median (IQR)	83	(78-86)	70	(65-77)
Sex				
Female	39	(63)	39	(60)
Male	23	(37)	26	(40)
Co-morbidities				
Hypertension	42	(68)	37	(57)
Missing	*0*	*(0)*	*0*	*(0)*
Ischaemic stroke	12	(19)	7	(11)
Missing	*0*	*(0)*	*0*	*(0)*
TIA	5	(8)	4	(6)
Missing	*0*	*(0)*	*1*	*(2)*
Dementia	10	(16)	12	(19)
Missing	*0*	*(0)*	*0*	*(0)*
Diabetes	6	(10)	6	(9)
Missing	*0*	*(0)*	*0*	*(0)*
Atrial Fibrillation	19	(31)	9	(14)
Missing	*0*	*(0)*	*0*	*(0)*
Hyperlipidaemia	8	(13)	18	(28)
Missing	*0*	*(0)*	*0*	*(0)*
Smoking status				
Current	11	(18)	5	(8)
Ex-smoker	23	(37)	19	(29)
Never	28	(45)	27	(42)
Missing	*0*	*(0)*	*14*	*(22)*
Medications on admission				
Antiplatelet drug(s)	33	(53)	16	(25)
Missing	*0*	*(0)*	*0*	*(0)*
Anticoagulant drug(s)	9	(15)	10	(15)
Missing	*0*	*(0)*	*0*	*(0)*
Admission GCS; median (IQR)	13	(9-14)	11	(8-14)
Missing	*0*	*(0)*	*19*	*(29)*
Time between ICH and diagnostic CT scan (days); median (IQR)	0	(0-1)	0	(0-0)
Tissue source				
Autopsy	62	(100)	26	(40)
Biopsy	0	(0)	39	(60)
Time between diagnostic CT scan and tissue (days); median (IQR)	11	(6-136)	2	(1-16)
CAA-associated lobar ICH	36	(58)	49	(75)
Death	62	(100)	44	(68)

Data are n (%) or median (IQR). * Relates to the largest ICH. APOE = Apolipoprotein E. CAA = cerebral amyloid angiopathy .CT = computed tomography. GCS = Glasgow coma scale. ICH = intracerebral haemorrhage.

Table 8.24 Non-contrast diagnostic brain CT characteristics in the Edinburgh CT and APOE criteria development versus external validation cohorts

	Development cohort (n=62)		External validation cohort (n=65)	
Multiple acute ICH	9	(15)	7	(11)
ICH location*				
Frontal	29	(47)	36	(55)
Parietal	14	(23)	6	(9)
Temporal	10	(16)	18	(28)
Occipital	9	(15)	5	(8)
Insular	0	(0)	0	(0)
ICH volume (ml)*; median (IQR)	60	(20-118)	55	(37-81)
Strictly lobar ICH	58	(94)	60	(92)
Intraventricular haemorrhage	31	(50)	25	(39)
Subarachnoid haemorrhage	43	(69)	53	(82)
Subdural haemorrhage	12	(19)	15	(23)
Finger-like projections*	14	(23)	25	(39)
Anterior white matter lucencies				
0	10	(16)	15	(23)
1	37	(60)	31	(48)
2	15	(24)	19	(29)
Posterior white matter lucencies				
0	13	(21)	20	(31)
1	9	(15)	12	(19)
2	40	(65)	33	(51
Central atrophy				
0	19	(31)	26	(40)
1	39	(63)	35	(54)
2	4	(6)	4	(6)
Cortical atrophy				
0	15	(24)	10	(15)
1	33	(53)	52	(80)
2	14	(23)	3	(5)

Data are n (%) or median (IQR). * Relates to the largest ICH. APOE = apolipoprotein E. CT = computed tomography. ICH = intracerebral haemorrhage. SVD = small vessel disease.

Table 8.25 Baseline clinical and non-contrast diagnostic brain CT characteristics in participants classified as CAA-associated versus non-CAA-associated ICH by the histopathological reference standard

	Non-CAA associated lobar ICH (n=16)		CAA associated lobar ICH (n=49)		p value
Age (years)	67	(60-75)	71	(66-78)	0.068
Sex					
Female	12	(75)	27	(55)	0.158
Male	4	(25)	22	(45)	
Co-morbidities					
Hypertension	10	(63)	22	(45)	0.604
Ischaemic stroke^	3	(19)	4	(8)	0.350
Transient ischaemic attack^	0	(0)	4	(8)	0.565
Dementia^	1	(6)	11	(22)	0.262
Diabetes^	2	(13)	4	(8)	0.631
Atrial Fibrillation^	2	(13)	7	(14)	1.000
Hyperlipidaemia^	4	(25)	14	(29)	1.000
Smoking status^					
Current	1	(9)	4	(10)	0.096
Ex-smoker	7	(64)	12	(30)	
Never	3	(27)	24	(60)	
Medications on admission					
Antiplatelet drug(s)^	2	(13)	14	(29)	0.318
Anticoagulant drug(s)^	2	(13)	8	(16)	1.000
Admission Glasgow Coma Scale	14	(7-15)	11	(8-14)	0.858
APOE ε2 possession	3	(19)	18	(37)	0.182
APOE ε4 possession	8	(50)	20	(41)	0.520
Time between ICH and diagnostic CT scan (days)	0	(0-0)	0	(0-0)	
Tissue source					
Autopsy	2	(13)	24	(49)	0.010
Biopsy	14	(88)	25	(51)	
Time between diagnostic CT scan and tissue (days)	1	(0-4)	4	(1-30)	0.069
Death	6	(38)	38	(78)	0.003

Data are n (%) or median (IQR). * Relates to the largest ICH. ^ Fisher's exact test. APOE = Apolipoprotein. CAA = cerebral amyloid angiopathy .CT = computed tomography. GCS = Glasgow coma scale. ICH = intracerebral haemorrhage.

Table 8.26 Non-contrast diagnostic brain CT characteristics in participants classified as CAA-associated versus non-CAA-associated ICH by the histopathological reference standard

	Non-CAA associated lobar ICH (n=16)		CAA associated lobar ICH (n=49)		p value
Multiple acute ICH^	2	(13)	5	(10)	1.000
ICH location*^					
Frontal	8	(5)	28	(57)	
Parietal	1	(6)	5	(10)	
Temporal	5	(31)	13	(27)	0.811
Occipital	2	(13)	3	(6)	
Insular	0	(0)	0	(0)	
ICH volume (ml)	49	(23-70)	54.7	(38-85)	0.433
Strictly lobar ICH	13	(81)	47	(96)	0.056
Intraventricular haemorrhage	6	(38)	19	(39)	0.927
Subarachnoid haemorrhage^	10	(63)	43	(88)	0.057
Subdural haemorrhage^	2	(25)	11	(22)	1.000
Finger-like projections	3	(19)	22	(45)	0.062
Anterior white matter lucencies ^					
0	7	(44)	8	(16)	
1	7	(44)	24	(49)	0.059
2	2	(13)	17	(35)	
Posterior white matter lucencies ^					
0	9	(56)	11	(22)	
1	1	(6)	11	(22)	0.040
2	6	(38)	27	(55)	
Central atrophy^					
0	10	(63)	16	(33)	
1	5	(31)	30	(61)	0.098
2	1	(6)	3	(6)	
Cortical atrophy^					
0	1	(6.2)	9	(18.4)	
1	15	(93.8)	37	(75.5)	0.431
2	0	(0.0)	3	(6.1)	
CT SVD score^					
0	8	(50)	17	(35)	
1	7	(44)	28	(57)	0.690
2	1	(6)	3	(6)	
3	0	(0)	1	(2)	

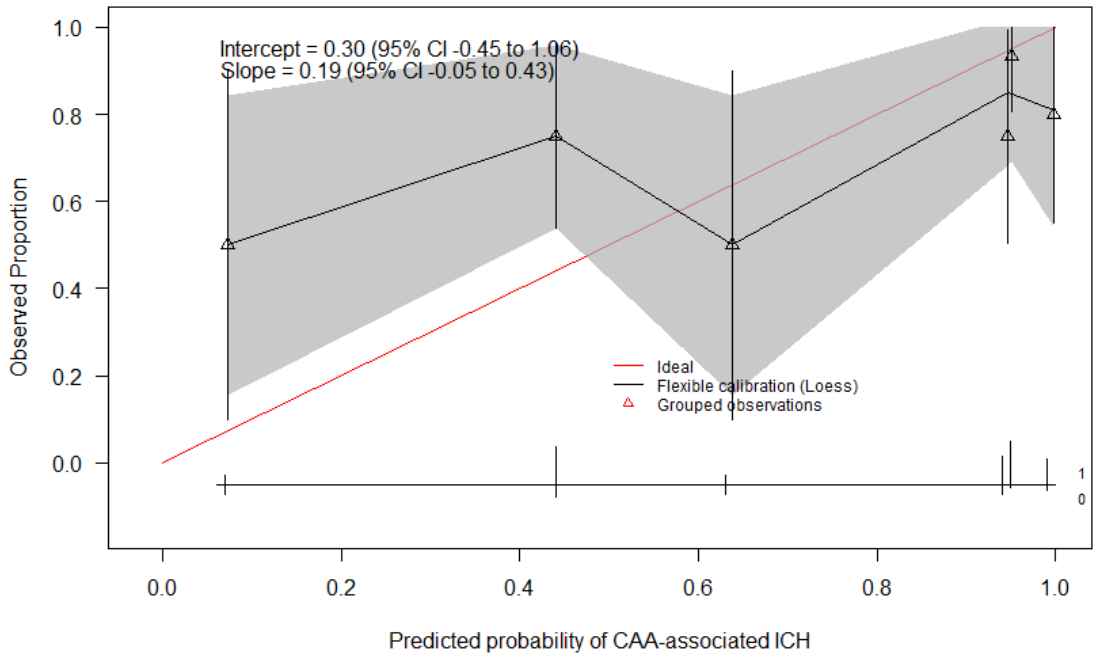
Data are n (%) or median (IQR). * Relates to the largest ICH. ^ Fisher's exact test. APOE = apolipoprotein E. CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. SVD = small vessel disease.

Participants with CAA-associated lobar ICH had more severe white matter lucencies than the non-CAA-associated lobar ICH group. CAA-associated participants were more likely to have undergone an autopsy. The median time between diagnostic CT scanning and tissue sampling was longer in the CAA-associated lobar ICH group, although this did not reach statistical significance.

8.4.2.5 Edinburgh CT and APOE diagnostic model performance

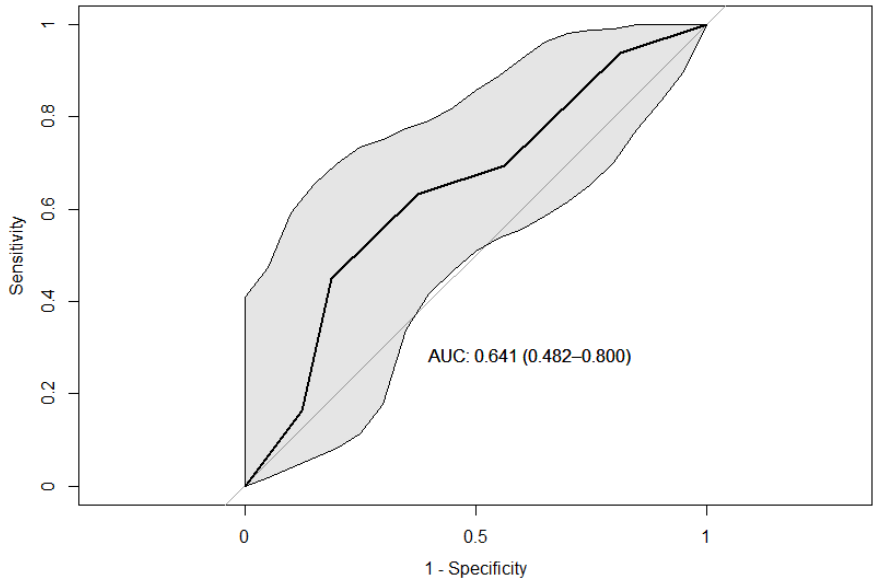
Figure 8.18 shows the model calibration. The model shows modest discrimination (c statistic 0.64, 95% CI 0.48 to 0.80, Figure 8.19). The net benefit of the model is shown in the decision curves (Figure 8.20).

Figure 8.18 Calibration plot of predicted probability of CAA-associated lobar ICH using the Edinburgh CT and APOE prediction model versus the observed frequency of CAA-associated lobar ICH.



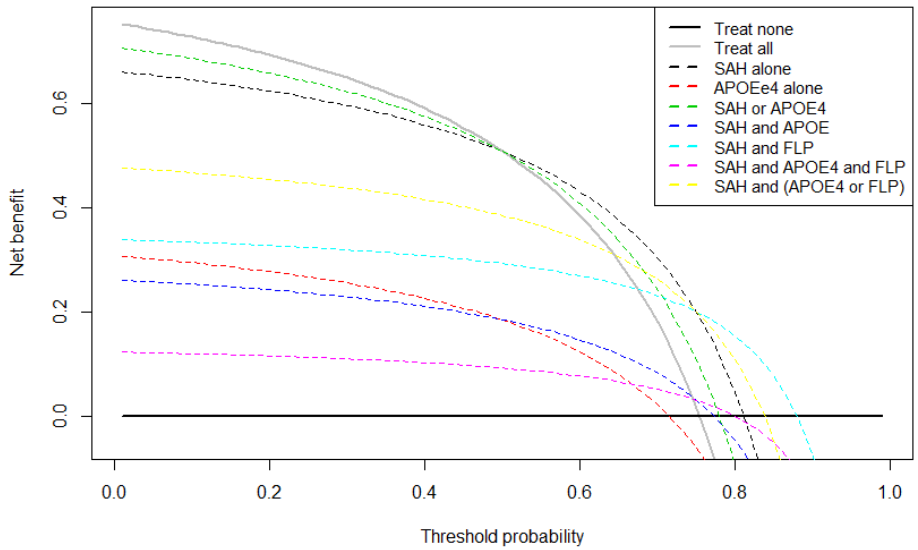
The red line indicates perfect calibration, the model's calibration is shown by the black line. The grey shaded area represents the 95%CI. Triangles represent the three different risk groups produced by the prediction model. Vertical lines at the bottom of the plot represent the distribution of model predicted probabilities stratified by endpoint (CAA-associated ICH above the x-axis, non-CAA-associated ICH below the x-axis). APOE = apolipoprotein E. CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage.

Figure 8.19 ROC curve for the predicted probability of CAA-associated lobar ICH using the Edinburgh CT and APOE prediction model.



The AUC is equivalent to the c statistic. The shaded area represents the 95% CI of the AUC based on 2000 bootstrap replicates. The grey line indicates a non-informative AUC of 0·50 for comparison. AUC = area under the curve. APOE = apolipoprotein E. CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. ROC = receiver operating characteristic.

Figure 8.20 Decision curves of predictions and classifications of CAA-associated lobar ICH using the Edinburgh CT and APOE prediction model



APOE = apolipoprotein E. CAA = cerebral amyloid angiopathy. CT = computed tomography. FLP = finger-like projection. ICH = intracerebral haemorrhage. SAH = subarachnoid haemorrhage.

8.4.2.6 Edinburgh CT and APOE criteria diagnostic accuracy

The cross-tabulation of Edinburgh CT and APOE diagnostic criteria against the reference standard is shown in Table 8.27. The presence of subarachnoid haemorrhage or APOE ε4 possession had a sensitivity of 94% (95% CI 82 to 98) for CAA-associated ICH (Table 8.28). The combination of subarachnoid haemorrhage and APOE ε4 possession and/or finger-like projections had a specificity of 63% (95% CI 36 to 84).

There were three false negative cases for the rule out criteria of subarachnoid haemorrhage or APOE ε4 possession (Table 8.27), two of which had an ICH volume less than 20ml and/or tissue sampling performed more than 1000 days after the ICH. There were six false positive cases for the rule in criteria of subarachnoid haemorrhage and APOE ε4 possession and/or finger-like projections, all of which were based on brain biopsy.

There were too few participants to do sensitivity analyses by the reference standard tissue source, ICH volume or time between CT and tissue sampling.

Table 8.27 Cross-tabulations of the Edinburgh CT and APOE diagnostic criteria for CAA-associated lobar ICH against the Vonsattel scale

Diagnostic criteria (index test) Subarachnoid haemorrhage or APOE ε4+	**Vonsattel grade ≥2 (Reference standard)** Positive	Negative	Total
Positive	46	13	59
Negative	3	3	6
Total	49	16	65
Diagnostic criteria (index test) Subarachnoid haemorrhage & (APOEε4+ and/or finger-like projections)	**Vonsattel grade ≥2 (Reference standard)** Positive	Negative	Total
Positive	31	6	37
Negative	18	10	28
Total	49	16	65

APOE = apolipoprotein E. CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage.

Table 8.28 Diagnostic accuracy statistics for the Edinburgh CT and APOE diagnostic criteria for CAA-associated lobar ICH in the development and external validation studies

	Development				External validation			
	Subarachnoid haemorrhage or APOE ε4+		**Subarachnoid haemorrhage & (APOEε4+ and/or finger-like projections)**		**Subarachnoid haemorrhage or APOE ε4+**		**Subarachnoid haemorrhage & (APOEε4+ and/or finger-like projections)**	
Sensitivity	100	(88-100)	64	(46-79)	94	(82-98)	63	(48-76)
Specificity	54	(34-73)	96	(78-100)	19	(5-46)	63	(36-84)
Positive likelihood ratio	2.2	(1.4-3.3)	16.6	(2.4-115)	1.2	(0.9-1.5)	1.7	(0.9-3.3)
Negative likelihood ratio	0	(0-NaN)	0.4	(0.2-0.6)	0.3	(0.1-1.7)	0.6	(0.4-0.9)
Positive predictive value	75	(60-86)	96	(77-100)	78	(65-87)	84	(67-93)
Negative predictive value	100	(73-100)	66	(49-80)	22	(13-35)	16	(7-33)

Data are percentage or ratio (95% confidence interval). APOE = apolipoprotein. CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. NaN = not a number – calculation cannot be performed because one of the values includes a zero

8.5 Discussion

8.5.1 Main findings

External validation of the Edinburgh CT-only diagnostic criteria and model

- Participants were younger than those I included in the Edinburgh CT diagnostic models and criteria development study. The predictors (subarachnoid haemorrhage and finger-like projections) and outcome (CAA-associated ICH) were more frequent than in the development setting.
- The frequency of CT predictors (subarachnoid haemorrhage and finger-like projections) was similar between those scanned on the day of symptom onset versus those scanned on day one onwards.
- Overall CAA-associated ICH was more frequent than predicted by the Edinburgh CT-only diagnostic model (intercept 0.15). The model shows good calibration for those with an intermediate predicted risk of CAA-associated ICH but underestimated the frequency in the low-risk group and over-estimated it in the high-risk group.
- The model shows good discrimination (c statistic 0.71)
- The Edinburgh CT-only rule out criteria of no subarachnoid haemorrhage or finger-like projections had a sensitivity of 88% compared with 89% in the development study.
- Edinburgh CT-only rule in criteria of subarachnoid haemorrhage and finger-like projections had a specificity of 84% compared with 100% in the development study.
- The sensitivity of the rule out criteria was lower in those with an autopsy reference standard, while the specificity of the rule in criteria was lower in those with a brain biopsy reference standard.
- The rule out criteria were most sensitive in those with an ICH volume above the median (56 ml) and the rule in criteria most specific in those with an ICH volume below the median.

External validation of the Edinburgh CT and APOE diagnostic criteria and model

- Participants were younger than those I included in the Edinburgh CT and APOE diagnostic models and criteria development study. The CT predictors (subarachnoid haemorrhage and finger-like projections) and outcome (CAA-associated ICH) were more frequent than in the development setting.
- The Edinburgh CT and APOE diagnostic model showed limited calibration, particularly for those with the lowest predicted risk of CAA-associated ICH, and modest discrimination (c statistic 0.64).
- The Edinburgh CT and APOE rule out criteria of no subarachnoid haemorrhage, APOE ε4 possession or finger-like projections were 94% sensitive compared with 100% in the development study
- The rule in criteria of subarachnoid haemorrhage and either APOE ε4 possession or finger-like projections had a specificity of 63% compared with 96% in the development study.

8.5.2 Strengths of the study

I performed and reported the study according to the TRIPOD guidelines for multivariable prediction models,[332] and the STARD guidelines for diagnostic accuracy studies.[173] Important strengths are:

- I identified as many research groups with relevant data as possible by liaising with the International CAA Association and systemically reviewing the literature.
- Participants were from different countries and healthcare settings, increasing the generalisability of the results.
- I only included participants with first-ever ICH to provide a standard inception point.
- I used both autopsy and brain biopsy as the reference standard to include younger participants with non-fatal ICH compared with the development cohort, increasing the generalisability of the results.
- I minimised information bias for predictor assessment by standardising imaging format, using the same definition for CT predictors as I used in the development study, undertaking online training for predictor

assessment before rating the CT scans, and performing the ratings masked to clinical, genetic and histopathological data.

- Reference standard assessment was performed by experienced neuropathologists masked to clinical, CT, genetic and outcome data, using a validated scale for CAA.[253] I pre-specified the reference standard definition.
- There were no missing predictor or outcome data for the external validation of the CT-only model and criteria.
- I described the baseline clinical and radiographic features and the distribution of CAA and non-CAA SVD severity in the study groups to describe the spectrum of participants.[175, 176]

8.5.3 Limitations of the study

The main limitations relate to selection bias and the time delay to obtaining the reference standard.

The sources of data I used introduced a selection bias. Participants were often part of hospital-based case series identified by collaborators rather than part of a community-based consecutive sample, as was the case with the development cohort. In most of the contributing cohorts the decision to perform APOE genotyping, and brain biopsy or autopsy was made by the treating medical team rather than being offered to all. In such cases, the decision to perform these procedures seemed to have been influenced by the clinical suspicion of CAA based on clinical history and imaging findings. This results in a partial verification bias, where the reference standard is preferentially performed in those most likely to have the outcome of interest. In line with this, the prevalence of subarachnoid haemorrhage, finger-like projection and CAA-associated ICH was higher in this external validation study compared with the development cohort. CAA-associated ICH was significantly more frequent in participants with APOE genotype (75%) compared to those without (59%). Therefore the external validation cohort is likely to be enriched for CAA-associated lobar ICH and not representative of

general clinical practice, particularly those with APOE genotype. This is likely to bias the results by falsely increasing the reported sensitivity of the criteria.

The time between the index test and tissue sampling for the reference standard was long for some participants (over a year in 16 participants, and over five years in eight). CAA is a progressive, age-related condition, so its presence in delayed tissue samples does not necessarily reflect the pathological changes at the time of the CT scan following the index ICH, leading to false positive reference standard classifications. The prevalence of CAA when the reference standard was obtained over one year after the index ICH was 88%, compared with 64% obtained within a year. Therefore some of the participants may have been misclassified by the reference standard.

There were several other limitations, which are likely to have smaller effects on the results.

Despite CAA and ICH being common and the source studies coming from research groups with special interest in CAA and ICH, the available sample size was relatively small. Therefore the study did not reach the pre-specified sample size of 100 cases and 100 controls. In particular, the CT and APOE external validation cohort only included 49 CAA-associated ICH cases and 16 non-CAA-associated ICH controls. This leads to a risk of a type II error. However, it was the largest sample I could achieve by approaching all potential research groups and using both autopsy and brain biopsy as the reference standard. In addition, the sample size of the CT-only external validation cohort is twice that of the development cohort.

Baseline data collection was performed retrospectively in some cohorts. However, the proportion of missing baseline data was low apart from admission GCS, which was missing in 25%. CT predictors and the outcome were available for all included participants.

I performed all CT ratings. Assessment by other raters from different centres and with different levels of experience is important to assess the inter-rater agreement of the CT predictors.

The reference standard I used was different to that used in the development cohort. In the development study, a single consultant neuropathologist performed systematic research autopsies and rated the tissue using a consensus CAA scale.[36] We classified a CAA-associated ICH where there was moderate or severe parenchymal CAA in the left cerebral hemisphere. In this study, I included both brain biopsy and autopsy in the reference standard. Brain biopsy is prone to sampling errors leading to false negative and false positive results compared with autopsy (Section 4.4.4).[164] Histopathological samples were assessed locally by experienced neuropathologists using the Vonsattel scale.[253] Participants were classified as having CAA-associated lobar ICH if there was Vonsattel grade ≥2 in any tissue sample. These factors may introduce variability in the classification of the reference standard between centres and with the development study. However, the Vonsattel CAA scale is a familiar, widely used scale. Furthermore, the cut-off of Vonsattel grade ≥2 correlates closely with the definition we used in the development study, even when applied in a biopsy sample (Section 4.4.4.6).

8.5.4 Study findings

8.5.4.1 External validation of the Edinburgh CT-only diagnostic model and criteria

The Edinburgh CT-only diagnostic model showed good calibration for intermediate predicted probability (subarachnoid haemorrhage) of CAA-associated lobar ICH (model predicted risk 62%, observed frequency 56%). The model underestimated the frequency of CAA-associated ICH for the low predicted risk cases (model predicted risk 23%, observed frequency 43%) and overestimated the frequency of CAA-associated ICH for high predicted risk cases (model predicted risk 97%, observed frequency 87%). The positive intercept (0.15) indicates that model predictions are systematically too low (calibration-in-the-large). The calibration slope (0.49) is less than 1, reflecting that the model predictions are too extreme (low predicted risk too low and vice versa).[336] The discriminative value of the model remained good (c statistic 0.71), although this was less than in the development setting (c

statistic 0.82). The combination of subarachnoid haemorrhage and finger-like projections maximises net benefit for high threshold probabilities (0.6-0.9) where false-positive results are to be avoided. For low threshold probabilities (0-0.4), the subarachnoid haemorrhage line is below the approach of considering all as having CAA-associated ICH, and is therefore not as useful for minimising false-negatives or ruling out CAA-associated ICH.

The rule out criterion of no subarachnoid haemorrhage showed similarly high sensitivity for CAA-associated ICH in the external validation setting (88% 95% CI 79 to 93) compared with the development study (89% 95% CI 73 to 96). The rule in criteria of subarachnoid haemorrhage and finger-like projections had a specificity of 84% (95% CI 70 to 92) compared with 100% (95% CI 84 to 100) in the development cohort.

Performance of a predictive model and diagnostic criteria in other settings depends on the quality of the prediction model, the characteristics of the external validation cohort and the accuracy of the reference standard.[334]

Prediction models can be overfitted during development. This is especially true if the development sample is small.[338, 339] Overfitting results in predictions that are too extreme (low predicted risk too low and vice versa) at external validation, reducing discrimination and clinical usefulness.[334] I developed the Edinburgh diagnostic criteria on a small cohort of 62 participants, so there is a chance the model is overfitted. To limit overfitting during model development, I only included two predictors in the CT-only model (13 events per variable). There was minimal optimism during internal validation, suggesting little overfitting.[340] I did not shrink the regression coefficients because the Firth's penalised likelihood logistic regression results in conservative coefficients. However, shrinkage of the regression coefficients would further reduce overfitting.[338]

Even if the model is not overfitted, there are likely to be true differences in regression coefficients between the development and external validation populations related to the case mix and outcome definition. The frequency of CAA-associated ICH was higher in the external validation population

compared with the development cohort. This may relate to partial verification bias, where selection of patients for tissue sampling favoured those with a higher clinical suspicion of CAA, and the outcome definition as only one tissue sample needed to be classified as Vonsattel ≥2 for the participant to be classified as CAA-associated lobar ICH. A higher proportion with the outcome increases the intercept and reduces the net benefit.[334] The effect of the outcome incidence on model performance is highlighted by the sensitivity analyses based on the source of tissue. The frequency of CAA-associated ICH in the autopsy group was 90%, and the intercept is high (1.81), whereas only 54% of the biopsy group were CAA-associated and the intercept was low (-0.40).

The frequency of subarachnoid haemorrhage and finger-like projections were higher in the external validation cohort compared with the development study, resulting in fewer participants with low predicted probabilities of CAA and more with high predicted probabilities of CAA. The mean predicted probability of CAA-associated ICH in the external validation cohort was higher (69%) than in the development setting (58%), whilst the spread of prediction was similar in both cohorts. This will have reduced the c statistic and the net benefit.[334] The spread of predicted probabilities was greater when I restricted the analyses to the autopsy cases, and there was an increase in the c statistic in this subgroup (0.83).

Assessment of diagnostic performance depends on accurate classification by the reference standard. The eight participants with a high predicted probability of CAA-associated ICH but classified as negative by the reference standard were all based on brain biopsy. Brain biopsy is prone to sampling bias and false negative results.[164] Five of the 12 participants with a low predicted risk but classified as positive by the reference standard had tissue sampling performed over 1000 days after the diagnostic CT scan. Delayed tissue sampling increases the chance of the interval development of CAA, and therefore a false positive histopathological assessment.

The presence of subarachnoid haemorrhage is related to ICH volume.[237] Finger-like projections were also more frequent in larger ICH volumes in both the development and external validation cohorts. I therefore assessed the performance of the diagnostic model and criteria stratifying by ICH volume. The model showed better calibration and discrimination when restricted to those with an ICH volume above the median (Figure 8.14 and Figure 8.15). The presence of subarachnoid haemorrhage was most sensitive for CAA-associated ICH (96%, 95% CI 85 to 99%) in those with an ICH volume above the median, suggesting the absence of subarachnoid haemorrhage in this group can confidently rule out CAA-associated ICH. Conversely, the sensitivity of subarachnoid haemorrhage in the low ICH volume group was 78% (95% CI 63 to 89). The presence of subarachnoid haemorrhage and finger-like projections was most specific in those with an ICH volume below the median (93%, 95% CI 75 to 99). Therefore the presence of these two features in this group can be used to rule in the diagnosis. In contrast, the specificity of these findings in the high ICH volume group was 71% (95% CI 48 to 88).

8.5.4.2 External validation of the Edinburgh CT and APOE diagnostic model and criteria

The Edinburgh CT and APOE diagnostic model showed reasonable calibration for the intermediate predicted probability (APOE ε4 possession) and high predicted probability of CAA-associated ICH. The model underestimated the frequency of CAA-associated ICH for the low predicted and intermediate (subarachnoid haemorrhage) probability cases. The positive intercept (0.30) indicates that model predictions are systematically too low (calibration-in-the-large). The calibration slope (0.19) is less than 1, reflecting the model predictions are too extreme.[336] The model showed only modest discrimination (c statistic 0.64).

The rule out criteria of no subarachnoid haemorrhage or APOE ε4 possession showed high sensitivity for CAA-associated ICH (94% 95% CI 82 to 98) compared with 100% (95% CI 88 to 100) in the development setting.

The rule in criteria of subarachnoid haemorrhage and APOE ε4 possession or finger-like projections had limited specificity (63%, 95% CI 36 to 84) compared with 96% (95% CI 78 to 100) in the development cohort.

As described above, there are several possible reasons for poor external validation of the model. In the CT and APOE external validation study, each of these is amplified.

Overfitting in the development study is possible given that I included three variables (8.7 events per variable) and did not shrink the regression coefficients. However, two of the variables were pre-specified, and there was minimal optimism after internal validation.

The frequency of CAA-associated ICH was much higher in the external validation (75%) compared to the development cohort (58%). This may relate to partial verification bias, where selection of patients for APOE genotyping favoured those with a higher clinical suspicion of CAA. This will increase the intercept and reduce net benefit.[334]

The frequency of predictors was also higher in the external validation cohort than in the development cohort. The mean predicted probability of CAA-associated ICH was 72% in the external validation cohort compared with 57% in the development cohort, with less spread of the predictions (standard deviation 31% versus 35% respectively). These factors will reduce the discrimination and net benefit of the model.[334]

There were few participants classified as non-CAA-associated lobar ICH, which will impact on the specificity of the rule out criteria.[176]

8.5.5 Clinical implications

This study assessed the external validity of the Edinburgh CT-only model in a heterogeneous group of participants. The CT-only criteria showed good sensitivity and specificity for ruling out and ruling in CAA-associated lobar ICH. The CT-only criteria were most sensitive for CAA-associated ICH in larger ICH (≥56 ml) and could be used to rule out CAA-associated ICH in this group reliably. The CT-only criteria were most specific for CAA-associated

ICH when applied to smaller ICH (<56 ml). Therefore, the criteria can be used to reliably rule in CAA-associated ICH in this setting. This potentially obviates the need for further investigations, such as MRI, β-amyloid PET or brain biopsy, which are more costly and less available than CT, and carry potential complications.

The external validation study of the Edinburgh CT and APOE model was small with significant partial verification bias. It is therefore difficult to draw firm conclusions.

8.5.6 Future directions

Larger studies with unbiased routes to data collection are needed to refine the estimates of the Edinburgh CT-only model performance and to determine the external validity of the Edinburgh CT and APOE model and criteria. Samples should ideally be unselected and representative to establish generalisability in clinical practice,[334] and include APOE genotyping where possible. APOE genotyping and tissue sampling (brain biopsy or autopsy) should be offered to all participants to reduce the effect of partial verification bias.

The current study included participants from Europe and North America. Assessment of the criteria in other geographical settings and participants of different ethnicities is needed to assess their wider generalisability.

The CT ratings need to be performed by raters of different experience to determine whether the criteria can be reliably implemented by others. Imaging data in this study are currently being rated by Dr Andreas Charidimou, a neurology trainee in Boston, to assess inter-rater agreement. I have also developed online training materials for the Edinburgh criteria to hopefully improve inter-rater agreement.[330] Users can log into the website and rate a series of cases, which allows rater-agreement from a diverse group of raters to be assessed.

Tissue sampling should be performed as close to the index ICH as possible, to limit the chance of false positive histopathological findings. The use of

systematically acquired autopsy provides the most robust reference standard, however, brain biopsy allows the criteria to be assessed in non-fatal ICH cases. Assessment of both CAA and non-CAA SVD is important, given that patients with ICH may have CAA in isolation or mixed with non-CAA SVD (Figure 4.13).[229] Differentiating these categories from pure non-CAA SVD may be informative for prognosis.

Finally, further work is needed to assess the impact of the Edinburgh criteria on clinical management and outcome of ICH patients.

Chapter 9 Diagnostic test accuracy studies of the Edinburgh diagnostic criteria for CAA-associated lobar ICH against the modified Boston criteria

9.1 Introduction

The MRI-based modified Boston criteria are the non-invasive *in vivo* reference standard for diagnosing CAA-associated ICH [110] and are often used in clinical practice when MRI is available and in CAA research.[167, 168] However, access to MRI may be limited in many parts of the world. Even in settings where MRI is available, many patients with ICH may not be able to tolerate an MRI scan. In the three-year community-based LATCH study of ICH in the NHS Lothian Health Board region, less than 30% of patients underwent a research brain MRI scan (Figure 3.4). Those who underwent MRI tended to be younger, with less pre-ICH disability and less frequent pre-ICH dementia, have a smaller ICH and less severe SVD features on CT. More widely available diagnostic tests for CAA-associated ICH are therefore needed.

The Edinburgh criteria for CAA-associated lobar ICH are based primarily on CT, which is the most widely available neuroimaging modality, and usually the first test performed to diagnose ICH (Figure 3.1). The criteria can also incorporate APOE genotype which is performed on a peripheral blood test and is therefore potentially available worldwide, although not currently used routinely in the clinical setting. The Edinburgh CT-only criteria showed good sensitivity and specificity for diagnosing CAA-associated lobar ICH in the development setting (Chapter 7, [229]) and in an external validation study (Chapter 8). The Edinburgh CT and APOE criteria had excellent sensitivity and specificity for CAA-associated lobar ICH in the development setting (Chapter 7, [229]). However, how the Edinburgh criteria relate to the more established modified Boston criteria is unknown.

9.2 Aims

I aimed to:

- Perform diagnostic test accuracy studies of the Edinburgh CT-only and CT-APOE diagnostic criteria for CAA-associated lobar ICH (index tests) against the MRI-based modified Boston criteria (reference standard).
- Compare the Edinburgh CT-only and CT-APOE criteria for CAA-associated lobar ICH and the modified Boston criteria against a histopathological reference standard.

9.3 Methods

9.3.1 Study design and participants

I used data from the prospective LINCHPIN study (section 2.1.3.2). I included consecutive adult participants (aged ≥16 years) living in the NHS Lothian Health Board region who had a first-ever lobar ICH between 1st June 2010 and 31st May 2016 inclusive diagnosed by non-contrast brain CT who had a subsequent research brain MRI scan. Some of the LINCHPIN participants were included in the development and external validation studies of the Edinburgh diagnostic criteria.

I defined lobar ICH as described in section 2.1.5.[190]

I excluded patients with exclusively extra-axial intracranial haemorrhage, non-lobar ICH, ICH secondary to an underlying cause other than SVDs, recurrent ICH and those without diagnostic quality non-contrast brain CT and research brain MRI with T2*-GRE sequence. I did not exclude participants used in the development or external validation of the Edinburgh criteria.

9.3.2 Baseline data collection

We collected demographics, the presence of relevant co-morbidities and medication use at the time of ICH as described in the section 2.1.7.

9.3.3 Index tests

I reformatted the first diagnostic non-contrast brain CT and assessed them using a standardised pro forma as described in Section 2.2. I performed brain CT assessments masked to clinical, genetic, MRI and pathological data.

APOE genotyping was performed on DNA extracted from peripheral blood or brain tissue using standard techniques described in Chapter 7, masked to radiological, clinical and pathological data. I defined APOE ε2 and APOE ε4 allele possession if participants had at least one ε2 allele or one ε4 allele respectively, as described in Chapter 7.

I pre-specified the low-risk Edinburgh criteria categories (CT-only: no subarachnoid haemorrhage or finger-like projections; CT-APOE: no subarachnoid haemorrhage, no APOE ε4 allele possession and no finger-like projections) as the negative index test results as these cut-offs maximise the sensitivity of the criteria, and are the most useful for ruling out CAA.

I pre-specified the high-risk Edinburgh criteria categories (CT-only: subarachnoid haemorrhage and finger-like projections; CT-APOE: subarachnoid haemorrhage plus APOE ε4 allele possession and/or finger-like projections) as the positive index test results as these cut-offs maximise the specificity of the criteria, and are the most useful for ruling in CAA.

9.3.4 Reference standards

9.3.4.1 MRI-based Modified Boston criteria

I used the modified Boston criteria as the reference standard because it is the current non-invasive *in vivo* reference standard for diagnosing CAA.[167] I pre-specified the “probable CAA” category as CAA-associated lobar ICH and “possible CAA” or “no CAA” categories as non-CAA-associated lobar ICH given the high sensitivity (95% 95%CI 83-99) and moderate specificity (81% 95%CI 62-93) of this cut-off for CAA.[110] Probable CAA is also the key diagnostic cut-off used in clinical practice and in research.[167]

I rated the research brain MRI scans for the presence of acute ICH and ischaemia, chronic ischaemic infarcts, lacunes of presumed vascular origin,

chronic haemorrhage and SVD biomarkers using the standardised pro forma described in the methods section (Appendix 2). I used the T2*-GRE sequence to categorise scans as probable CAA, possible CAA or no CAA according to the modified Boston criteria.[110] I performed all assessments masked to clinical, CT, genetic and pathological data. I performed the MRI ratings at least six months after the corresponding diagnostic non-contrast brain CT assessment to reduce diagnostic-review bias.[176]

9.3.4.2 Research autopsy

Where available, I compared the Edinburgh CT and modified Boston criteria against a research brain autopsy reference standard. Research brain autopsies were performed within five days of death according to a standard operating procedure. [217] A single neuropathologist (Professor C Smith) assessed samples for CAA severity using a consensus scale developed by Love et al[36] as described in Section 4.3.3.[229] I classified lobar ICH cases as CAA-associated using two cut-offs. First, if there was moderate or severe parenchymal CAA in the left cerebral hemisphere on the Love et al consensus scale[36] as used in the development of the Edinburgh criteria (Section 4.3.3 and Chapter 7).[229] And second, if the severity of CAA in any tissue sample was ≥2 on the Vonsattel scale as used in the development of the modified Boston criteria (Section 8.3.5).[110, 253]

9.3.5 Statistical analysis

I compared the frequency of clinical, genetic, CT and MRI characteristics between groups using χ^2 test (or Fisher's exact test, where appropriate) for categorical variables and the Mann-Whitney U test for non-normally distributed continuous variables.

To determine the performance of the Edinburgh CT-only and CT-APOE diagnostic criteria I classified participants as low, intermediate or high risk of moderate/severe CAA using the cut-offs defined in the development study (chapter 7).[229] I assessed the diagnostic accuracy of the Edinburgh CT-only and CT and APOE rule in criteria (high versus low/intermediate risk groups) and rule out (low versus intermediate/high risk) criteria separately

against the probable CAA category on the modified Boston criteria reference standard using sensitivity, specificity, likelihood ratios, and predictive values and their 95% CI.

I performed statistical analyses using R statistical package version 3.4.4., except for the diagnostic accuracy statistics, for which I used VassarStats Clinical Calculator 1.[269]

9.3.6 Missing data

I excluded any participants with missing APOE ε4 genotype from the CT and APOE criteria analysis. I did not to impute these missing values as APOE ε4 is one of only three predictors in the Edinburgh CT and APOE diagnostic model. I excluded participants with missing or poor quality research MRI scans as this was the reference standard, and I felt it was inappropriate to impute any missing data.

9.3.7 Sample size

I was unable to calculate the sample size required for a diagnostic test accuracy study because no studies have previously compared the Edinburgh and modified Boston criteria. Therefore, I used the largest sample possible from the prospective LINCHPIN study.

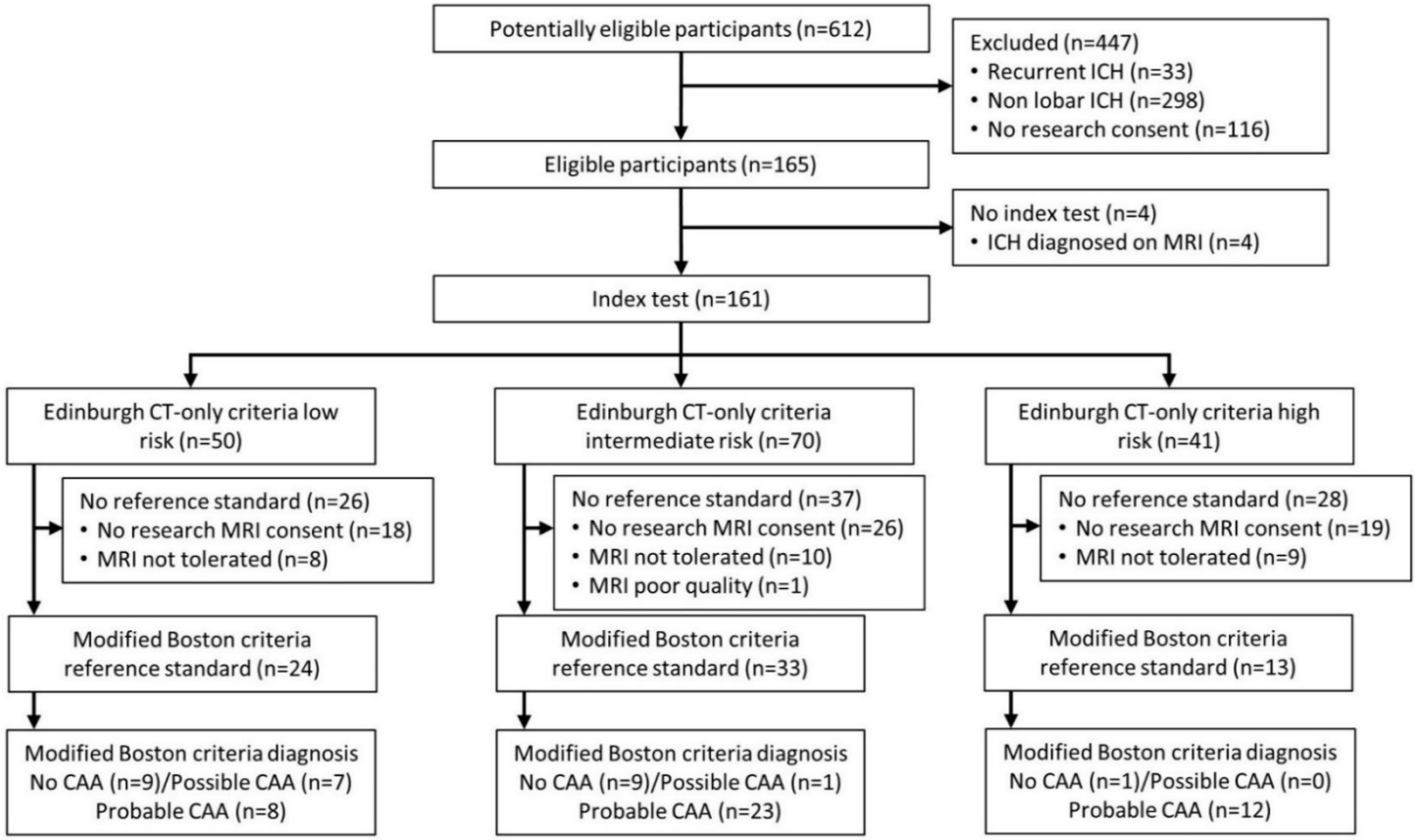
9.4 Results

9.4.1 Edinburgh CT-only criteria versus the modified Boston criteria diagnostic test accuracy study

9.4.1.1 Flow of participants

There were 612 patients with spontaneous ICH presumed related to SVD between 1st June 2010 and 31st May 2016. One hundred and sixty-five patients with a first-ever lobar ICH consented to the LINCHPIN study, of whom 161 had a diagnostic non-contrast brain CT. There were no reported adverse effects with the index test. I included 70 participants who had a subsequent research brain MRI with T2*-GRE sequence (Figure 9.1).

Figure 9.1 Flow of participants through the Edinburgh CT-only versus modified Boston criteria diagnostic test accuracy study



CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. MRI = magnetic resonance imaging. Edinburgh CT-only CAA criteria: Low risk = no subarachnoid haemorrhage or finger-like projections; Intermediate risk = subarachnoid haemorrhage or finger-like projections; High risk = subarachnoid haemorrhage and finger-like projections.

9.4.1.2 Comparison of participants with the index test who underwent the MRI-based reference standard versus those who did not

Participants who underwent both the index test (diagnostic non-contrast brain CT) and reference standard (research brain MRI) were younger with less frequent pre-ICH dementia, atrial fibrillation and ischaemic stroke and had a lower median pre-ICH modified Rankin scale score compared with LINCHPIN participants who only had the index test (Table 9.1). Those with both the index test and reference standard also had higher admission GCS, a smaller ICH volume, less frequent intraventricular haemorrhage and a lower CT SVD score compared with those who only had the index test (Table 9.2). The frequency of subarachnoid haemorrhage and finger-like projections was also lower in those who underwent both the index test and reference standard, although these differences did not reach statistical significance.

Table 9.1 Baseline clinical features of first-ever lobar ICH participants with a diagnostic non-contrast brain CT (index test) who also had a research brain MRI (reference standard) versus those who did not

	Index test only (n=91)		**Index test and reference standard (n=70)**		**p value**
Age (years); median (IQR)	82	(78-86)	77	(67-81)	<0.001
Sex					
Female	54	(59)	42	(60)	0.933
Male	37	(41)	28	(40)	
Co-morbidities					
Hypertension	58	(64)	35	(50)	0.080
Ischaemic stroke	13	(14)	2	(3)	0.013
Transient ischaemic attack	11	(12)	3	(4)	0.082
Dementia	16	(18)	1	(1)	0.001
Diabetes	10	(11)	6	(9)	0.611
Atrial fibrillation	25	(28)	10	(14)	0.044
Myocardial infarction	11	(12)	3	(4)	0.082
Hyperlipidaemia	13	(14)	5	(7)	0.154
Smoking status					
Current	13	(14)	15	(21)	
Ex-smoker	34	(37)	19	(27)	0.288
Never	44	(48)	36	(51)	
Pre-ICH modified Rankin scale; median (IQR)	2	(2-4)	1	(1-1)	<0.001
Medications on admission					
Antiplatelet drug(s)	46	(51)	24	(34)	0.039
Anticoagulant drug(s)	16	(18)	9	(13)	0.412
Antihypertensive drug(s)	53	(58)	27	(39)	0.013
Admission GCS; median (IQR)	12	(10-14)	15	(14-15)	<0.001

Data are n (%) or median (IQR). * Fisher's exact test. CT = computed tomography. GCS = Glasgow coma scale. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. MRI = magnetic resonance imaging.

Table 9.2 Non-contrast diagnostic brain CT features of first-ever lobar ICH participants with a diagnostic non-contrast brain CT (index test) who also had a research brain MRI (reference standard) versus those who did not

	Index test only (n=91)		**Index test and reference standard (n=70)**		**p value**
ICH volume (ml); median (IQR)	55	(20-95)	18	(7-33)	<0.001
Intraventricular haemorrhage	42	(46)	10	(14)	<0.001
Subarachnoid haemorrhage	65	(71)	46	(66)	0.437
Subdural haemorrhage	20	(22)	9	(13)	0.135
Finger-like projections	28	(31)	13	(19)	0.078
CT SVD score*					
0	23	(25)	39	(56)	<0.001
1	48	(53)	20	(29)	
2	16	(18)	11	(16)	
3	4	(4)	0	(0)	
CT-only CAA category					
Low	26	(29)	24	(34)	0.211
Intermediate	37	(41)	33	(47)	
High	28	(31)	13	(19)	

Data are n (%) or median (IQR). * Fisher's exact test. CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. MRI = magnetic resonance imaging. SVD = small vessel disease.

9.4.1.3 Baseline clinical and diagnostic non-contrast brain CT characteristics in CAA-associated and non-CAA-associated lobar ICH groups

Forty-three participants were classified as probable CAA on the modified Boston criteria reference standard ("CAA-associated lobar ICH") while 27 were classified as "non-CAA-associated lobar ICH" (8 possible CAA and 19 no CAA). The frequency of co-morbidities and antithrombotic drug use and the time between diagnostic CT and research MRI were similar between the groups (Table 9.3). Subarachnoid haemorrhage and finger-like projections were significantly more frequent in the CAA-associated lobar ICH participants compared with the non-CAA-associated group (Table 9.4).

Table 9.3 Baseline clinical features of participants classified as CAA-associated versus non-CAA-associated lobar ICH by the modified Boston criteria

	Non-CAA-associated lobar ICH (n=27)		**CAA-associated lobar ICH (n=43)**		**p value**
Age (years); median (IQR)	75	(56-81)	77	(71-81)	0.207
Sex					
Female	13	(48)	29	(67)	0.109
Male	14	(52)	14	(33)	
Co-morbidities					
Hypertension	14	(52)	21	(49)	0.806
Ischaemic stroke*	1	(4)	1	(2)	1.000
Transient ischaemic attack*	0	(0)	3	(7)	0.279
Dementia*	0	(0)	1	(2)	1.000
Diabetes*	1	(4)	5	(12)	0.394
Atrial fibrillation*	5	(19)	5	(12)	0.493
Myocardial infarction*	2	(7)	1	(2)	0.555
Hyperlipidaemia*	0	(0)	5	(12)	0.149
Smoking status					
Current	4	(15)	11	(26)	
Ex-smoker	7	(26)	12	(28)	0.485
Never	16	(59)	20	(47)	
Pre-ICH modified Rankin scale; median (IQR)	1	(1-2)	1	(1-1)	0.539
Medications on admission					
Antiplatelet drug(s)	8	(30)	16	(37)	0.515
Anticoagulant drug(s)*	4	(15)	5	(12)	0.726
Antihypertensive drug(s)	11	(41)	16	(37)	0.768
Admission GCS; median (IQR)	15	(14-15)	15	(14-15)	0.415
Days between index ICH & CT; median (IQR)	1	(0-2)	1	(0-2)	0.727
Days between CT & MRI; median (IQR)	74	(66-105)	92	(73-118)	0.466

Data are n (%) or median (IQR). * Fisher's exact test. CAA = cerebral amyloid angiopathy. CT = computed tomography. GCS = Glasgow coma scale. ICH = intracerebral haemorrhage. MRI = magnetic resonance imaging.

Table 9.4 Non-contrast diagnostic brain CT features of participants classified as CAA-associated versus non-CAA-associated lobar ICH by the modified Boston criteria

	Non-CAA-associated lobar ICH (n=27)		**CAA-associated lobar ICH (n=43)**		**p value**
ICH volume (ml); median (IQR)	14	(3-22)	19	(14-34)	0.106
Intraventricular haemorrhage*	5	(19)	5	(12)	0.493
Subarachnoid haemorrhage	11	(41)	35	(81)	<0.001
Subdural haemorrhage*	1	(4)	8	(19)	0.139
Finger-like projections	1	(4)	12	(28)	0.011
CT SVD score*					
0	14	(52)	25	(58)	
1	8	(30)	12	(28)	0.840
2	5	(19)	6	(14)	
3	0	(0)	0	(0)	
CT-only CAA category*					
Low	16	(59)	8	(19)	
Intermediate	10	(37)	23	(54)	<0.001
High	1	(4)	12	(28)	

Data are n (%) or median (IQR). * Fisher's exact test. CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. SVD = small vessel disease.

9.4.1.4 Distribution of MRI SVD-biomarkers between CAA-associated and non-CAA-associated lobar ICH groups

Cortical superficial siderosis was present in 37 out of 43 participants with CAA-associated lobar ICH, compared with 11 out of 27 with non-CAA-associated lobar ICH (Table 9.5). Participants with CAA-associated lobar ICH also had a significantly higher median MRI CAA SVD burden score. There was no statistically significant difference in the severity of WMH, atrophy or PVS between the groups. The presence and number of lobar CMBs were also similar between the groups.

Table 9.5 MRI characteristics of participants classified as CAA-associated versus non-CAA-associated lobar ICH by the modified Boston criteria

	Non-CAA-associated lobar ICH (n=27)	CAA-associated lobar ICH (n=43)	p value
Periventricular Fazekas score; median (IQR)	3 (1-3)	3 (2-3)	0.401
Deep Fazekas score; median (IQR)	2 (1-2)	2 (1-2)	0.062
Central atrophy; median (IQR)	3 (2-4)	3 (2-4)	0.629
Cortical atrophy; median (IQR)	2 (1-3)	2 (1-3)	0.333
Basal ganglia PVS; median (IQR)	1 (1-2)	1 (1-2)	0.111
Centrum semiovale PVS; median (IQR)	2 (1-3)	2 (2-3)	0.425
Cortical superficial siderosis	11 (41)	37 (86)	<0.001
Any lobar CMB	10 (37)	17 (40)	0.834
Lobar CMBs; median (IQR)	0 (1-2)	0 (0-2)	0.772
Any deep CMB*	12 (44)	0 (0)	<0.001
Deep CMBs; median (IQR)	0 (0-1)	0 (0-0)	<0.001
Any cerebellar CMB*	2 (7)	3 (7)	1.000
Cerebellar CMBs; median (IQR)	0 (0-0)	0 (0-0)	0.946
Any brainstem CMB*	3 (11)	0 (0)	0.053
Brainstem CMBs; median (IQR)	0 (0-0)	0 (0-0)	0.027
Any CMB	12 (44)	19 (44)	0.983
Total CMBs; median (IQR)	0 (0-4)	0 (0-2)	0.609
MRI SVD burden score; median (IQR)	2 (1-3)	2 (1-2)	0.466
MRI CAA SVD burden score; median (IQR)	1 (1-2)	2 (1-4)	0.007

Data are n (%) or median (IQR). * Fisher's exact test. CAA = cerebral amyloid angiopathy. CMB = cerebral microbleed. CT = computed tomography. ICH = intracerebral haemorrhage. MRI = magnetic resonance imaging. PVS = perivascular space. SVD = small vessel disease.

9.4.1.5 Diagnostic accuracy of the Edinburgh CT-only criteria against the modified Boston criteria

Table 9.6 shows the cross-tabulation of the Edinburgh CT-only criteria (index test) against the modified Boston criteria classification (reference standard). Thirty-five out of the 43 participants with CAA-associated lobar ICH had subarachnoid haemorrhage on CT, resulting in a sensitivity of 81% (95% CI 66-91, Table 9.7) for the rule out Edinburgh CT-only criteria. One out of 28 non-CAA-associated lobar ICH had both subarachnoid haemorrhage and finger-like projections on the Edinburgh CT-only criteria, resulting in a specificity of 96% (95%CI 79-100) for the rule in Edinburgh CT-only criteria.

Table 9.6 Cross-tabulations of the Edinburgh CT-only criteria classifications against the modified Boston criteria

Edinburgh CT-only criteria (Index test)	**Modified Boston criteria (Reference standard)**			
	No CAA	Possible CAA	Probable CAA	Total
Low	9	7	8	24
Intermediate	9	1	23	33
High	1	0	12	13
Total	19	8	43	70

Data are number. CAA = cerebral amyloid angiopathy. CT = computed tomography. MRI = magnetic resonance imaging.

Table 9.7 Diagnostic test accuracy statistics for the Edinburgh CT-only criteria using probable CAA on the modified Boston criteria as the reference standard cut off

	Edinburgh CT-only criteria	
	Subarachnoid haemorrhage (Intermediate or High risk)	Subarachnoid haemorrhage and finger-like projections (High risk)
Sensitivity	81 (66-91)	24 (13-38)
Specificity	59 (40-77)	95 (72-100)
Positive likelihood ratio	2.0 (1.2-3.2)	4.5 (0.6-32.1)
Negative likelihood ratio	0.3 (0.2-0.6)	0.8 (0.7-0.9)
Positive predictive value	76 (61-87)	92 (62-100)
Negative predictive value	67 (45-84)	32 (20-45)

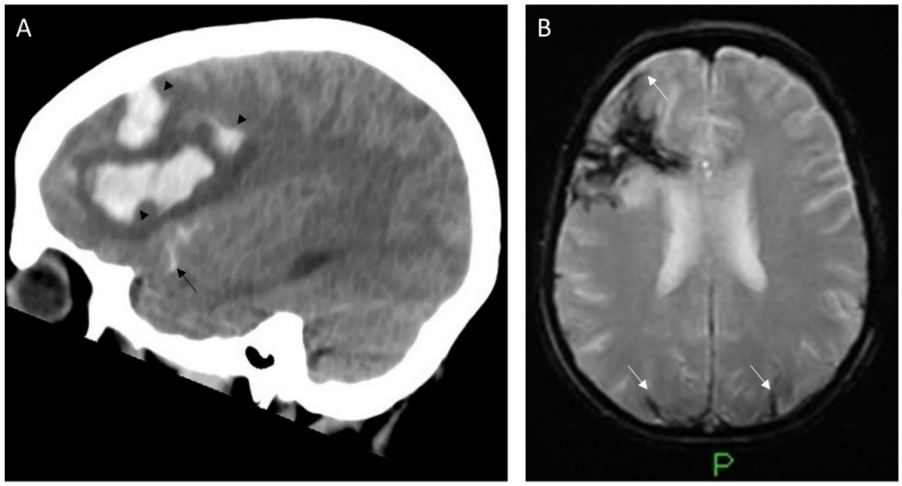
Data are percentage or ratio (95%CI). CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage.

Examples of true positive and true negative cases are shown in Figure 9.2 and Figure 9.3 respectively. The clinical and imaging findings for the one false positive case for the rule in criteria and the eight false negative cases for the rule out criteria are summarised in Figure 9.4 and Figure 9.5 respectively.

Figure 9.2 True positive result for the Edinburgh CT-only criteria against the modified Boston criteria

A. Sagittal non-contrast diagnostic brain CT image showing a right frontal ICH with finger-like projections (black arrowheads) and subarachnoid haemorrhage (black arrow). The participant is classified as high risk for CAA on the Edinburgh CT-only criteria.

B. Axial T2*-GRE image showing a right frontal ICH with diffuse cortical superficial siderosis (white arrows). The participant is classified as probable CAA on the modified Boston criteria.

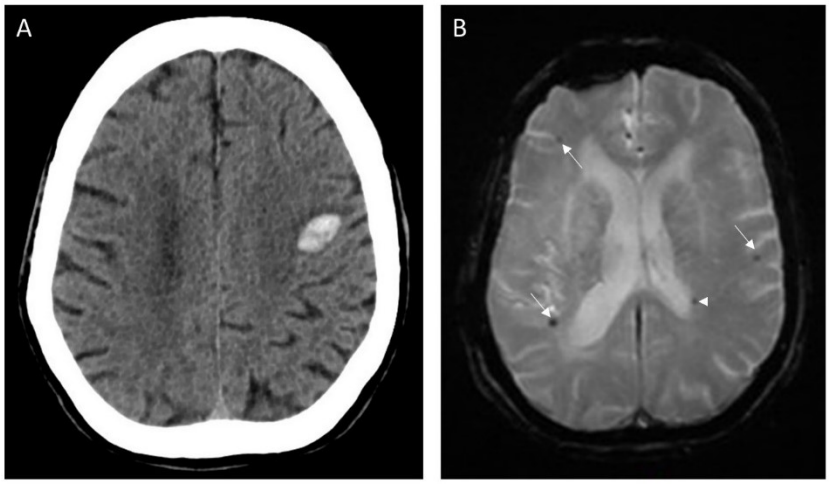


CAA = cerebral amyloid angiopathy. CT = computed tomography. GRE = gradient recalled echo. ICH = intracerebral haemorrhage. MRI = magnetic resonance imaging.

Figure 9.3 An example of a true negative index test result for the Edinburgh CT-only criteria against the MRI-based modified Boston criteria reference standard.

A. Axial non-contrast diagnostic brain CT image showing a small left frontal ICH. There is no subarachnoid haemorrhage or finger-like projections. The participant is classified as low risk for CAA on the Edinburgh CT-only criteria.

B. Axial T2*-GRE image showing lobar (white arrows) and deep (white arrowhead) cerebral microbleeds. The participant is classified as no CAA on the modified Boston criteria.

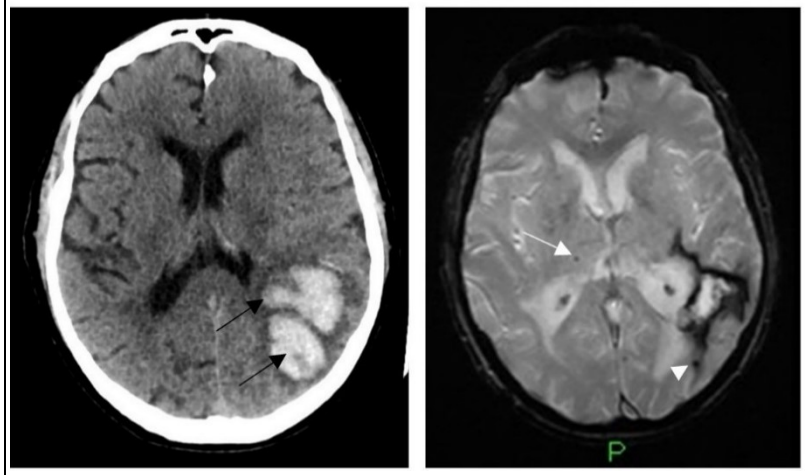


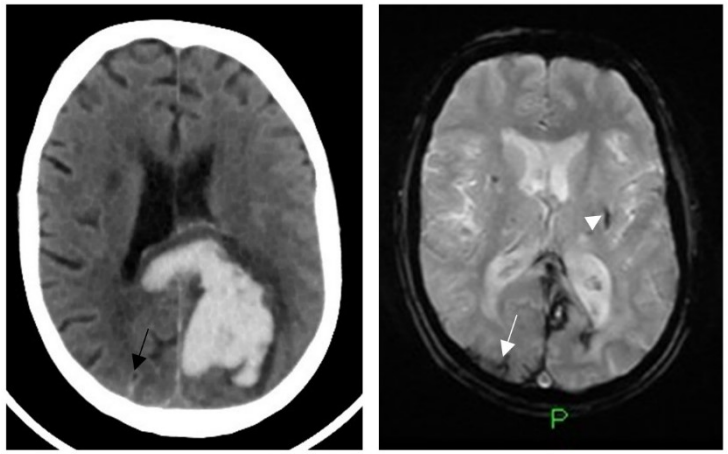
CAA = cerebral amyloid angiopathy. CT = computed tomography. GRE = gradient recalled echo. ICH = intracerebral haemorrhage. MRI = magnetic resonance imaging.

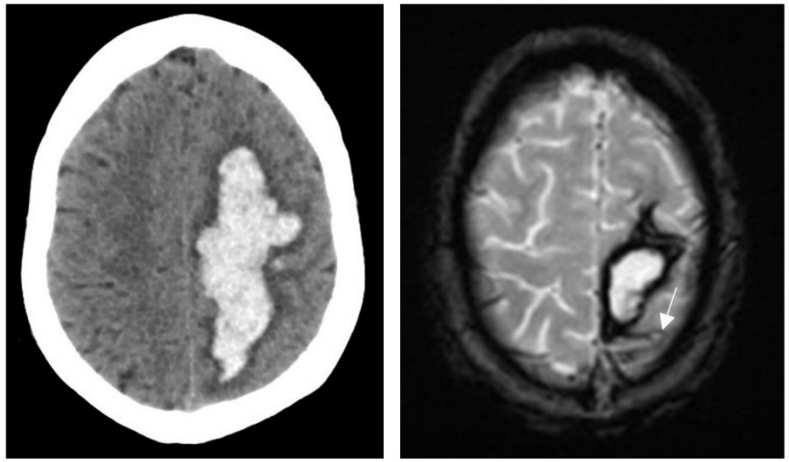
Figure 9.4 Discrepancies between the Edinburgh CT-only and CT-APOE criteria and the modified Boston criteria reference standard

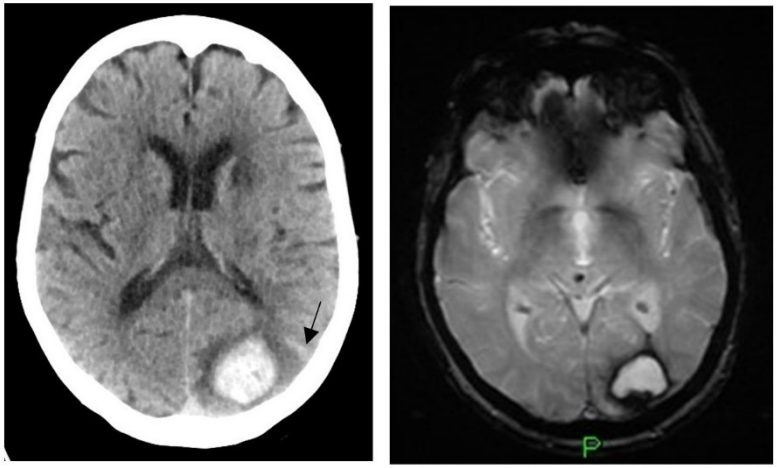
A. False positive index test result for both the Edinburgh CT-only and CT-APOE criteria.

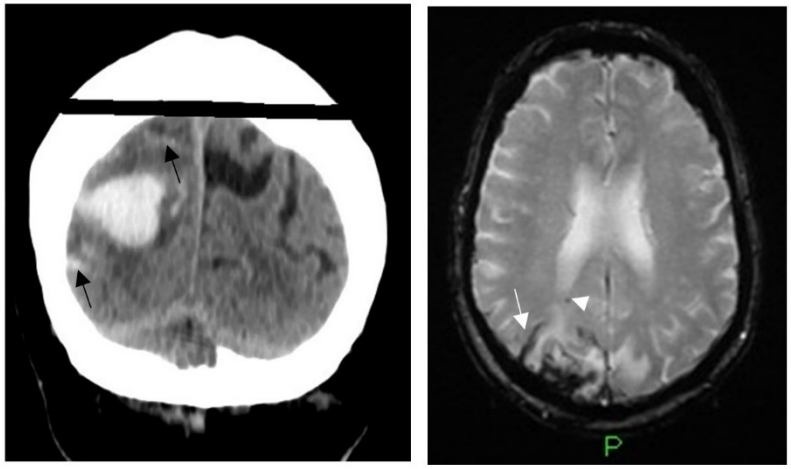
B to E. False positive index test results for the Edinburgh CT-APOE criteria alone.

	Clinical details	CT features	MRI features	Example CT and T2*-GRE images
A	78 year-old male Pre-ICH history of hypertension No pre-ICH antiplatelet or anticoagulant drug use APOE genotype ε3/4 Time from ICH to CT 0 days	Left parietal lobar ICH Subarachnoid haemorrhage Finger-like projections (black arrows) Edinburgh CT-only classification – High Edinburgh CT-APOE classification – High	Left parietal lobar ICH Focal cortical superficial siderosis Lobar microbleeds (white arrowhead) One deep microbleed (white arrow) Modified Boston criteria – No CAA Time from CT to MRI 105 days	

	Clinical details	CT features	MRI features	Example CT and T2*-GRE images
B	43 year-old male Pre-ICH history of hypertension No pre-ICH antiplatelet or anticoagulant drug use APOE genotype ε3/4 Time from ICH to CT 1 days	Left parietal lobar ICH Subarachnoid haemorrhage (black arrow) No finger-like projections Edinburgh CT-only classification – Intermediate Edinburgh CT-APOE classification – High	Left parietal lobar ICH, left lentiform nucleus (white arrowhead) ICH Diffuse cortical superficial siderosis (white arrow) No microbleeds Modified Boston criteria – No CAA Time from CT to MRI 140 days	

	Clinical details	CT features	MRI features	Example CT and T2*-GRE images
C	50 year-old female Pre-ICH history of hypertension No pre-ICH antiplatelet or anticoagulant drug use APOE genotype ε3/4 Time from ICH to CT 0 days	Left frontal lobar ICH Subarachnoid haemorrhage No finger-like projections Edinburgh CT-only classification – Intermediate Edinburgh CT-APOE classification – High	Left frontal lobar ICH Focal cortical superficial siderosis (white arrow) No microbleeds Modified Boston criteria – No CAA Time from CT to MRI 73 days	

	Clinical details	CT features	MRI features	Example CT and T2*-GRE images
D	82 year-old female No pre-ICH history of hypertension Pre-ICH anticoagulant drug use (INR 2.5) APOE genotype ε2/4 Time from ICH to CT 0 days	Left occipital lobar ICH Subarachnoid haemorrhage (black arrow) No finger-like projections Edinburgh CT-only classification – Intermediate Edinburgh CT-APOE classification – High	Left occipital lobar ICH No cortical superficial siderosis No microbleeds Modified Boston criteria – Possible CAA Time from CT to MRI 61 days	

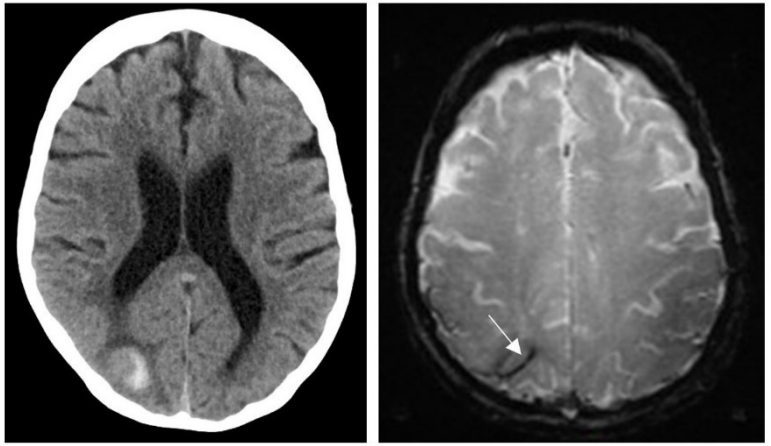
	Clinical details	CT features	MRI features	Example CT and T2*-GRE images
E	83 year-old male Pre-ICH history of hypertension Pre-ICH antiplatelet drug use APOE genotype ε3/4 Time from ICH to CT 1 days	Right occipital lobar ICH Subarachnoid haemorrhage (black arrows) No finger-like projections Edinburgh CT-only classification – Intermediate Edinburgh CT-APOE classification – High	Right occipital lobar ICH Focal cortical superficial siderosis (white arrow) Lobar and deep (white arrowhead) microbleeds Modified Boston criteria – No CAA Time from CT to MRI 91 days	

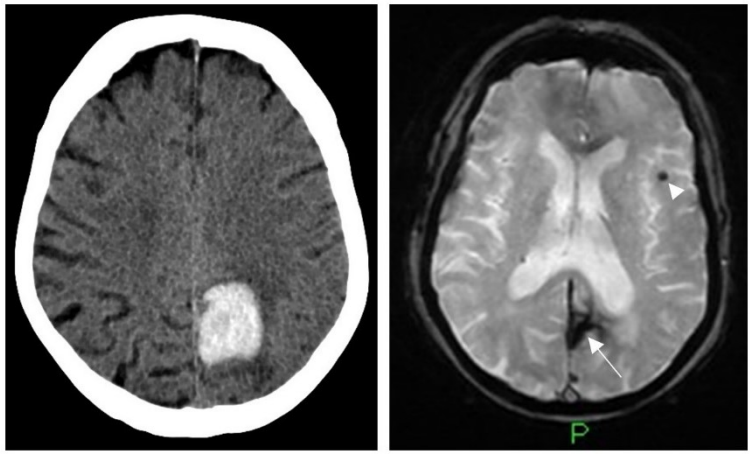
APOE = apolipoprotein E. CAA = cerebral amyloid angiopathy. CT = computed tomography. GRE = gradient recalled echo. ICH = intracerebral haemorrhage. INR = international normalised ratio. MRI = magnetic resonance imaging.

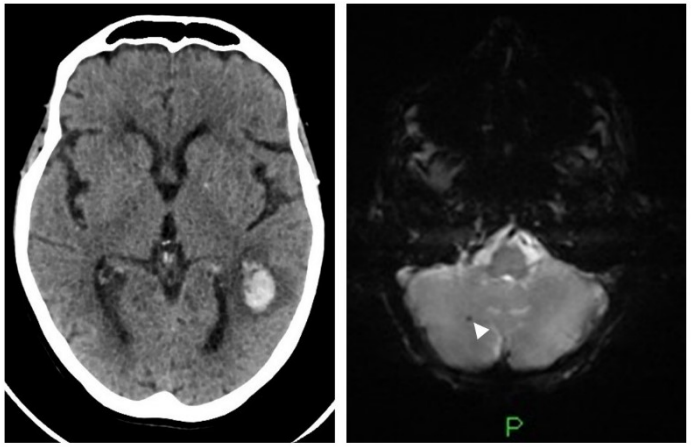
Figure 9.5 Discrepancies between the Edinburgh CT-only and CT-APOE criteria and the modified Boston criteria reference standard

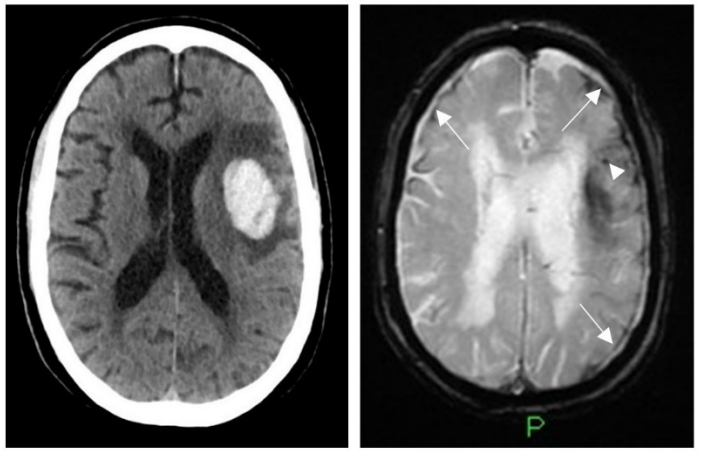
A to E. False negative index test results for the Edinburgh CT-only criteria alone.

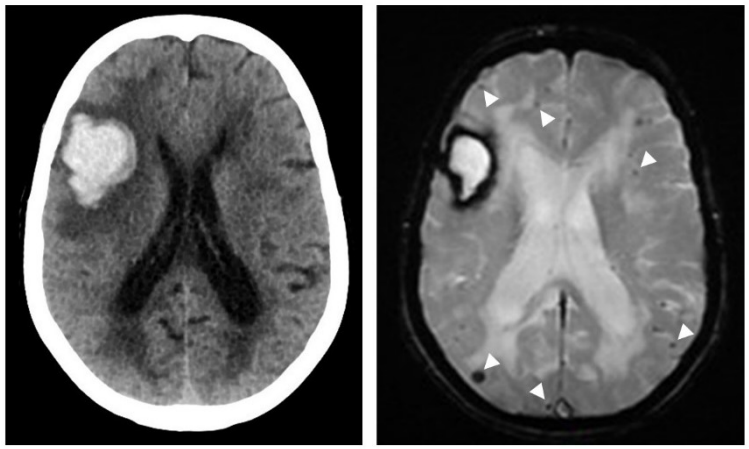
F to H. False negative index test results for both the Edinburgh CT-only and CT-APOE criteria.

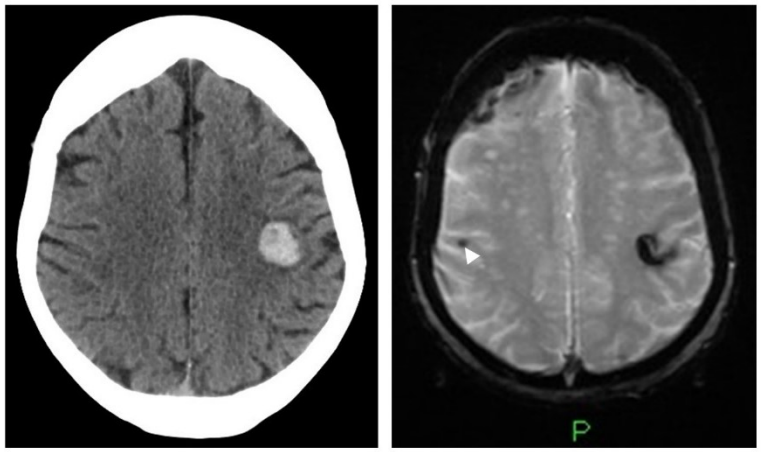
	Clinical details	CT features	MRI features	Example CT and T2*-GRE images
A	74 year-old female Pre-ICH history of hypertension Pre-ICH antiplatelet drug use APOE genotype ε2/4 Time from ICH to CT 5 days	Right occipital lobar ICH No subarachnoid haemorrhage No finger-like projections ICH volume 3 ml Edinburgh CT-only classification – Low Edinburgh CT-APOE classification - Intermediate	Right occipital lobar ICH Focal cortical superficial siderosis (white arrow) No microbleeds Modified Boston criteria – Probable CAA Time from CT to MRI 91 days	

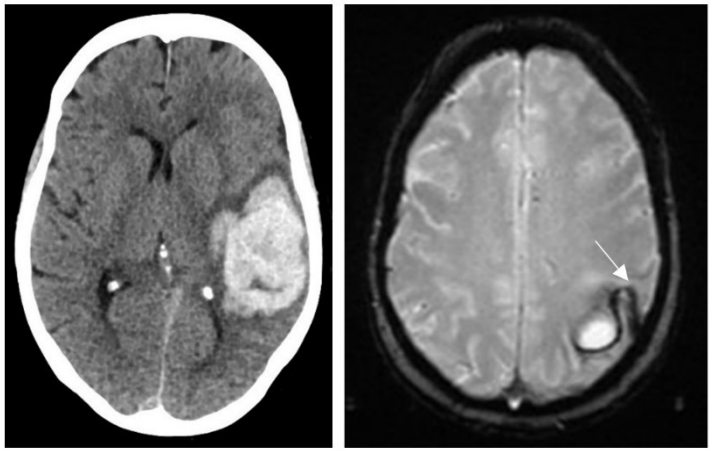
	Clinical details	CT features	MRI features	Example CT and T2*-GRE images
B	79 year-old female Pre-ICH history of hypertension Pre-ICH anticoagulant drug use (INR 3.0) APOE genotype ε4/4 Time from ICH to CT 3 days	Left parietal lobar ICH No subarachnoid haemorrhage No finger-like projections ICH volume 20 ml Edinburgh CT-only classification – Low Edinburgh CT-APOE classification - Intermediate	Left parietal lobar ICH Focal cortical superficial siderosis (white arrow) Lobar microbleeds (white arrowhead) Modified Boston criteria – Probable CAA Time from CT to MRI 95 days	

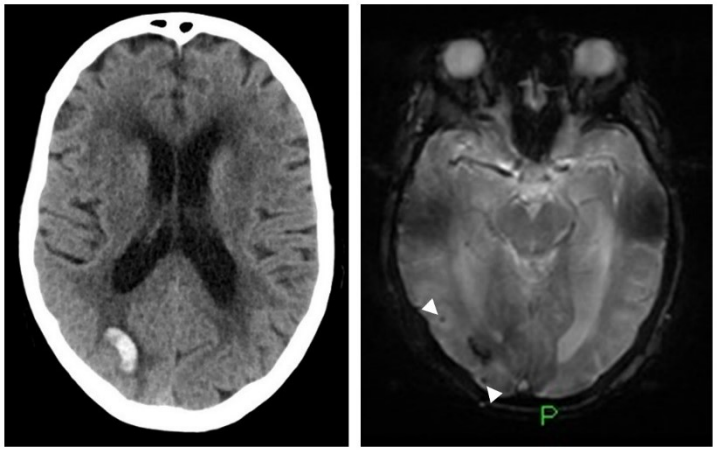
	Clinical details	CT features	MRI features	Example CT and T2*-GRE images
C	83 year-old female Pre-ICH history of hypertension Pre-ICH antiplatelet drug use APOE genotype ε3/3 Time from ICH to CT 0 days	Left temporal lobar ICH No subarachnoid haemorrhage No finger-like projections ICH volume 5 ml Edinburgh CT-only classification – Low Edinburgh CT-APOE classification - Low	Left temporal lobar ICH No cortical superficial siderosis Cerebellar microbleeds (white arrowhead) Modified Boston criteria – Probable CAA Time from CT to MRI 123 days	

	Clinical details	CT features	MRI features	Example CT and T2*-GRE images
D	81 year-old male Pre-ICH history of hypertension Pre-ICH antiplatelet drug use APOE genotype ε3/4 Time from ICH to CT 1 days	Left frontal lobar ICH No subarachnoid haemorrhage No finger-like projections ICH volume 11 ml Edinburgh CT-only classification – Low Edinburgh CT-APOE classification - Intermediate	Left temporal lobar ICH Diffuse cortical superficial siderosis (white arrows) Lobar microbleed (white arrowhead) Modified Boston criteria – Probable CAA Time from CT to MRI 88 days	

	Clinical details	CT features	MRI features	Example CT and T2*-GRE images
E	80 year-old female Pre-ICH history of hypertension Pre-ICH antiplatelet drug use APOE genotype ε3/4 Time from ICH to CT 1 days	Right frontal lobar ICH No subarachnoid haemorrhage No finger-like projections ICH volume 20 ml Edinburgh CT-only classification – Low Edinburgh CT-APOE classification - Intermediate	Right frontal and left cerebellar ICHs Focal cortical superficial siderosis Multiple lobar microbleeds (white arrowheads) Modified Boston criteria – Probable CAA Time from CT to MRI 74 days	

	Clinical details	CT features	MRI features	Example CT and T2*-GRE images
F	74 year-old female Pre-ICH history of hypertension No pre-ICH antiplatelet or anticoagulant drug use APOE genotype ε3/3 Time from ICH to CT 0 days	Left frontal lobar ICH No subarachnoid haemorrhage No finger-like projections ICH volume 2 ml Edinburgh CT-only classification – Low Edinburgh CT-APOE classification - Low	Left frontal lobar ICH No cortical superficial siderosis Lobar microbleed (white arrowhead) Modified Boston criteria – Probable CAA Time from CT to MRI 85 days	

	Clinical details	CT features	MRI features	Example CT and T2*-GRE images
G	68 year-old female Pre-ICH history of hypertension No pre-ICH antiplatelet or anticoagulant drug use APOE genotype ε3/3 Time from ICH to CT 0 days	Left temporal lobar ICH No subarachnoid haemorrhage No finger-like projections ICH volume 50 ml Edinburgh CT-only classification – Low Edinburgh CT-APOE classification - Low	Left temporal lobar ICH Focal cortical superficial siderosis (white arrow) No microbleeds Modified Boston criteria – Probable CAA Time from CT to MRI 87 days	

	Clinical details	CT features	MRI features	Example CT and T2*-GRE images
H	82 year-old female No pre-ICH history of hypertension Pre-ICH antiplatelet drug use APOE genotype ε3/3 Time from ICH to CT 4 days	Right occipital lobar ICH No subarachnoid haemorrhage No finger-like projections ICH volume 4 ml Edinburgh CT-only classification – Low Edinburgh CT-APOE classification - Low	Right occipital lobar ICH No cortical superficial siderosis Lobar microbleeds (white arrowheads) Modified Boston criteria – Probable CAA Time from CT to MRI 31 days	

APOE = apolipoprotein E. CAA = cerebral amyloid angiopathy. CT = computed tomography. GRE = gradient recalled echo. ICH = intracerebral haemorrhage. MRI = magnetic resonance imaging.

9.4.2 Edinburgh CT-APOE criteria versus the modified Boston criteria

9.4.2.1 Flow of participants

One hundred and thirty-two of the 165 LINCHPIN participants with a first-ever lobar ICH consented to the LINCHPIN study and had both a diagnostic non-contrast brain CT and APOE genotyping. There were no reported adverse effects with the index tests. I included 58 participants who had a subsequent research brain MRI with T2*-GRE sequence (Figure 9.6).

9.4.2.2 Comparison of participants with the index test who underwent the reference standard versus those who did not

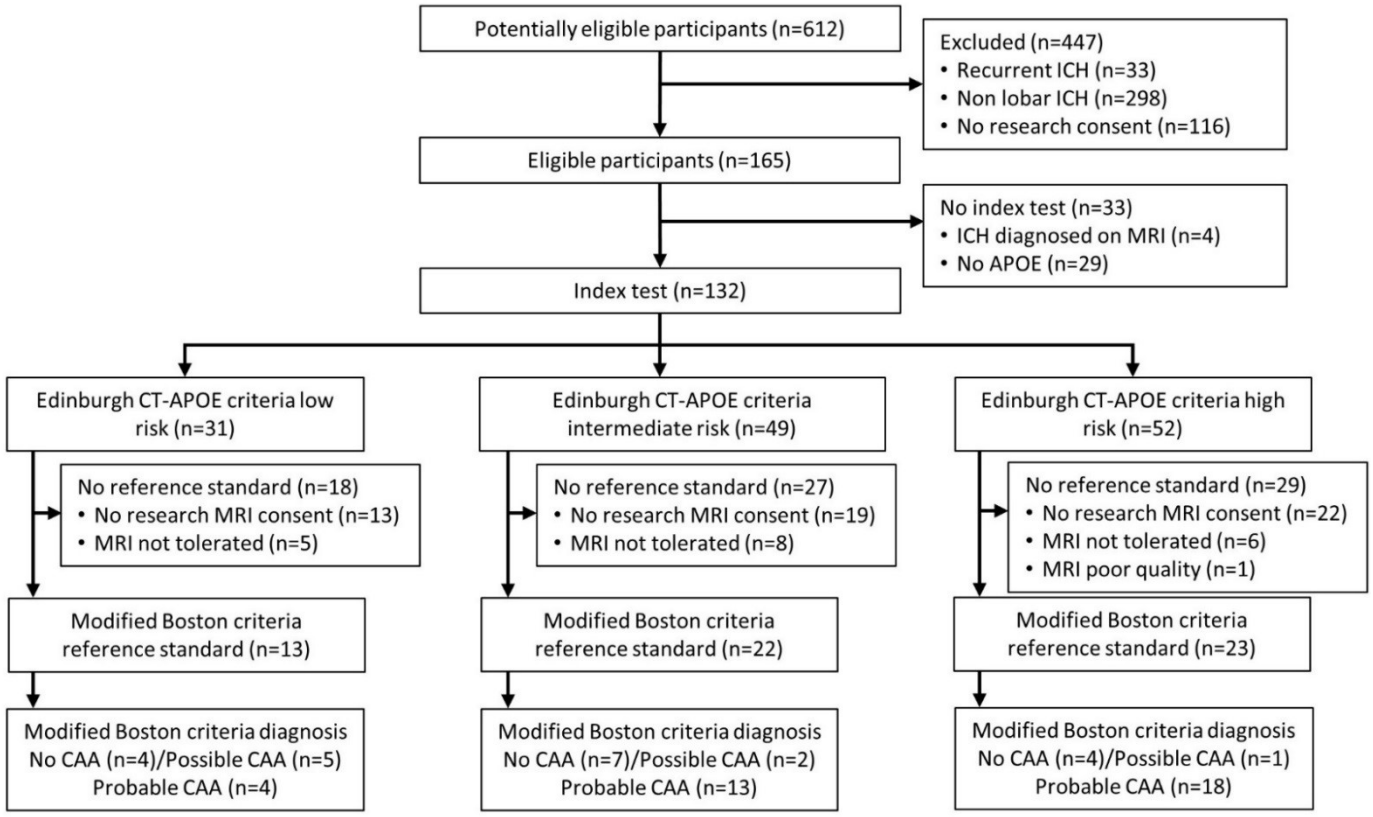
Participants who underwent both the index tests (diagnostic non-contrast brain CT and APOE genotyping) and reference standard (research brain MRI) were younger with less frequent pre-ICH dementia, atrial fibrillation, hypertension and ischaemic stroke and a lower median pre-ICH modified Rankin scale score compared with those who had only the index test (Table 9.8). Those with both the index tests and reference standard also had higher admission GCS, a smaller ICH volume, less frequent intraventricular and subdural haemorrhage and a less severe CT SVD score compared with those who had only the index test (Table 9.9). The frequencies of subarachnoid haemorrhage and finger-like projections were lower in those who underwent both the index test and reference standard, while APOE $\varepsilon 2$ and APOE $\varepsilon 4$ allele possession were more common, although these differences did not reach statistical significance.

9.4.2.3 Baseline clinical and diagnostic non-contrast brain CT characteristics associated with CAA-associated and non-CAA-associated lobar ICH groups

Thirty-five participants were classified as probable CAA on the modified Boston criteria reference standard (“CAA-associated lobar ICH”), whereas 23 were classified as “non-CAA-associated lobar ICH” (8 possible CAA and 15 no CAA). The frequency of co-morbidities and antithrombotic drug use and the time between diagnostic CT and research MRI were similar between the

groups (Table 9.10). Subarachnoid haemorrhage was significantly more frequent in the CAA-associated lobar ICH participants compared with the non-CAA-associated lobar ICH group (Table 9.11). Finger-like projections were also more common in the CAA-associated lobar ICH group, although this did not reach statistical significance.

Figure 9.6 Flow of participants in the Edinburgh CT-APOE criteria versus modified Boston criteria diagnostic test accuracy study



APOE = apolipoprotein E. CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. MRI = magnetic resonance imaging. Edinburgh CT & APOE CAA criteria: Low risk = no subarachnoid haemorrhage, APOE ε4 allele possession or finger-like projections; Intermediate risk = either subarachnoid haemorrhage or APOE ε4 allele possession; High risk = subarachnoid haemorrhage plus APOE ε4 allele possession and/or finger-like projections.

Table 9.8 Baseline clinical features of first-ever lobar ICH participants with a diagnostic non-contrast brain CT scan and APOE genotyping (index test) who had a research brain MRI (reference standard) versus those who did not

	Index test only (n=74)		Index test and reference standard (n=58)		p value
Age (years); median (IQR)	82	(77-86)	76	(67-80)	<0.001
Sex					
Female	43	(58)	37	(64)	0.507
Male	31	(42)	21	(36)	
Co-morbidities					
Hypertension	51	(69)	29	(50)	0.027
Ischaemic stroke	12	(16)	1	(2)	0.006
Transient ischaemic attack	10	(14)	3	(5)	0.110
Dementia	13	(18)	1	(2)	0.003
Diabetes	8	(11)	5	(9)	0.675
Atrial fibrillation	22	(30)	8	(14)	0.030
Myocardial infarction	11	(15)	2	(3)	0.029
Hyperlipidaemia	12	(16)	3	(5)	0.047
Smoking status					
Current	12	(16)	15	(26)	
Ex-smoker	27	(37)	16	(28)	0.321
Never	35	(47)	27	(47)	
Pre-ICH modified Rankin scale; median (IQR)	2	(2-4)	1	(1-1)	<0.001
Medications on admission					
Antiplatelet drug(s)	40	(54)	19	(33)	0.015
Anticoagulant drug(s)	11	(15)	8	(14)	0.862
Antihypertensive drug(s)	42	(57)	21	(36)	0.019
Admission GCS; median (IQR)	12	(9-14)	15	(14-15)	<0.001

Data are n (%) or median (IQR). * Fisher's exact test. APOE = apolipoprotein E. CT = computed tomography. GCS = Glasgow coma scale. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. MRI = magnetic resonance imaging.

Table 9.9 Non-contrast diagnostic brain CT features and APOE genotype of first-ever lobar ICH participants with a diagnostic non-contrast brain CT scan and APOE genotyping (index test) who had a research brain MRI (reference standard) versus those who did not

	Index test only (n=74)		Index test and reference standard (n=58)		p value
ICH volume (ml); median (IQR)	57	(20-101)	19	(7-33)	<0.001
Intraventricular haemorrhage	36	(49)	10	(17)	<0.001
Subarachnoid haemorrhage	51	(69)	37	(64)	0.535
Subdural haemorrhage	16	(22)	5	(9)	0.043
Finger-like projections	19	(26)	9	(16)	0.157
CT SVD score*					
0	17	(23)	34	(59)	
1	39	(53)	15	(26)	<0.001
2	14	(19)	9	(16)	
3	4	(5)	0	(0)	
APOE ε2 allele possession	17	(23)	20	(35)	0.144
APOE ε4 allele possession	23	(31)	26	(45)	0.105
CT-APOE CAA category					
Low	18	(24)	13	(22)	
Intermediate	27	(37)	22	(38)	0.965
High	29	(39)	23	(40)	

Data are n (%) or median (IQR). * Fisher's exact test. APOE = apolipoprotein E. CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. MRI = magnetic resonance imaging. SVD = small vessel disease.

Table 9.10 Baseline clinical features in participants classified as CAA-associated versus non-CAA-associated lobar ICH by the modified Boston criteria

	Non-CAA-associated lobar ICH (n=23)		**CAA-associated lobar ICH (n=35)**		**p value**
Age (years); median (IQR)	75	(56-80)	76	(70-81)	0.262
Sex					
Female	12	(52)	25	(71)	0.136
Male	11	(48)	10	(29)	
Co-morbidities					
Hypertension	12	(52)	17	(49)	0.788
Ischaemic stroke*	0	(0)	1	(3)	1.000
Transient ischaemic attack*	0	(0)	3	(9)	0.270
Dementia*	0	(0)	1	(3)	1.000
Diabetes*	0	(0)	5	(14)	0.146
Atrial fibrillation*	4	(17)	4	(11)	0.700
Myocardial infarction*	1	(4)	1	(3)	1.000
Hyperlipidaemia*	0	(0)	3	(9)	0.270
Smoking status					
Current	4	(17)	11	(31)	
Ex-smoker	6	(26)	10	(29)	0.386
Never	13	(57)	14	(40)	
Pre-ICH modified Rankin scale; median (IQR)	1	(1-2)	1	(1-1)	0.719
Medications on admission					
Antiplatelet drug(s)	6	(26)	13	(37)	0.380
Anticoagulant drug(s)*	4	(17)	4	(11)	0.700
Antihypertensive drug(s)	9	(39)	12	(34)	0.707
Admission GCS; median (IQR)	15	(14-15)	15	(14-15)	0.596
Days between index ICH & CT; median (IQR)	1	(0-2)	1	(0-2)	0.987
Days between CT & MRI ; median (IQR)	79	(66-121)	91	(76-110)	0.793

Data are n (%) or median (IQR). * Fisher's exact test. APOE = apolipoprotein E. CAA = cerebral amyloid angiopathy. CT = computed tomography. GCS = Glasgow coma scale. ICH = intracerebral haemorrhage. MRI = magnetic resonance imaging.

Table 9.11 Non-contrast diagnostic brain CT features and APOE genotype in participants classified as CAA-associated versus non-CAA-associated lobar ICH by the modified Boston criteria reference standard

	Non-CAA-associated lobar ICH (n=23)		**CAA-associated lobar ICH (n=35)**		**p value**
ICH volume (ml); median (IQR)	17	(4-35)	20	(13-33)	0.386
Intraventricular haemorrhage*	5	(22)	5	(14)	0.496
Subarachnoid haemorrhage	10	(44)	27	(77)	0.009
Subdural haemorrhage*	0	(0)	5	(14)	0.146
Finger-like projections*	1	(4)	8	(23)	0.073
CT SVD score*					
0	12	(52)	22	(63)	
1	6	(26)	9	(26)	0.606
2	5	(22)	4	(11)	
3	0	(0)	0	(0)	
APOE ε2 allele possession	8	(35)	12	(34)	0.969
APOE ε4 allele possession	9	(39)	17	(49)	0.479
CT-APOE CAA category					
Low	9	(39)	4	(11)	
Intermediate	9	(39)	13	(37)	0.020
High	5	(22)	18	(51)	

Data are n (%) or median (IQR). * Fisher's exact test. APOE = apolipoprotein E. CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. SVD = small vessel disease.

9.4.2.4 Distribution of MRI SVD-biomarkers between probable CAA and no CAA/possible CAA participants

Cortical superficial siderosis was present in 30 out of 35 participants with CAA-associated lobar ICH, compared with 9 out of 23 with non-CAA-associated lobar ICH (Table 9.12). Participants with CAA-associated lobar ICH also had a higher median MRI CAA SVD burden score. There was no statistically significant difference in the severity of WMH, atrophy or PVS between the groups. The presence and number of lobar CMBs were also similar between the groups.

Table 9.12 MRI characteristics of participants classified as CAA-associated versus non-CAA-associated lobar ICH by the modified Boston criteria reference standard.

	Non-CAA-associated lobar ICH (n=23)	CAA-associated lobar ICH (n=35)	P value
Periventricular Fazekas score; median (IQR)	3 (2-4)	4 (3-4)	0.274
Deep Fazekas score; median (IQR)	2 (2-3)	3 (2-3)	0.083
Central atrophy; median (IQR)	3 (2-4)	3 (2-4)	0.812
Cortical atrophy; median (IQR)	2 (2-3)	2 (1-3)	0.249
Basal ganglia PVS; median (IQR)	1 (1-2)	1 (1-2)	0.174
Centrum semiovale PVS; median (IQR)	3 (2-4)	3 (3-4)	0.272
Cortical superficial siderosis	9 (39)	30 (86)	<0.001
Any lobar CMB	8 (35)	11 (31)	0.790
Lobar CMBs; median (IQR)	0 (0-2)	0 (0-1)	0.717
Any deep CMB*	9 (39)	0 (0)	<0.001
Deep CMBs; median (IQR)	0 (0-1)	0 (0-0)	<0.001
Any cerebellar CMB*	2 (9)	3 (9)	1.000
Cerebellar CMBs; median (IQR)	0 (0-0)	0 (0-0)	0.987
Any brainstem CMB*	2 (9)	0 (0)	0.153
Brainstem CMBs; median (IQR)	0 (0-0)	0 (0-0)	0.078
Any CMB	9 (39)	13 (37)	0.879
Total CMBs; median (IQR)	0 (0-3)	0 (0-1)	0.471
MRI SVD burden score; median (IQR)	2 (1-3)	2 (1-2)	0.492
MRI CAA SVD burden score; median (IQR)	1 (0-2)	2 (1-3)	0.006

Data are n (%) or median (IQR). * Fisher's exact test. APOE = apolipoprotein E. CAA = cerebral amyloid angiopathy. CMB = cerebral microbleed. CT = computed tomography. MRI = magnetic resonance imaging. PVS = perivascular space. SVD = small vessel disease.

9.4.2.5 Diagnostic accuracy of the Edinburgh CT-APOE criteria against the modified Boston criteria

Table 9.13 shows the cross-tabulation of the Edinburgh CT-APOE criteria (index test) against the modified Boston criteria classification (reference standard). Thirty-one out of the 35 participants classified as CAA-associated lobar ICH had subarachnoid haemorrhage or APOE ε4 allele possession, resulting in a sensitivity of 89% (95% CI 72-96, Table 9.14) for the rule out Edinburgh CT-APOE criteria. Five out of 23 participants classified as non-CAA-associated lobar ICH had subarachnoid haemorrhage plus APOE ε4 allele possession and/or finger-like projections on the Edinburgh CT-APOE criteria, resulting in a specificity of 78% (95%CI 56-92) for the rule in Edinburgh CT-APOE criteria.

Table 9.13 Cross-tabulations of the Edinburgh CT-APOE criteria classifications against the modified Boston criteria

Edinburgh CT-APOE criteria (Index test)	**Modified Boston criteria (Reference standard)**			
	No CAA	Possible CAA	Probable CAA	Total
Low	4	5	4	13
Intermediate	7	2	13	22
High	4	1	18	23
Total	15	8	35	58

Data are number. APOE = apolipoprotein E. CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. MRI = magnetic resonance imaging.

Table 9.14 Diagnostic test accuracy statistics for the Edinburgh CT-APOE criteria using probable CAA on the modified Boston criteria as the reference standard cut off

	Edinburgh CT-APOE criteria	
	Subarachnoid haemorrhage or APOE ε4+ (Intermediate or High risk	Subarachnoid haemorrhage and (APOE ε4+ or finger-like projections) (High risk)
Sensitivity	89 (72-96)	51 (34-68)
Specificity	39 (20-61)	78 (56-92)
Positive likelihood ratio	1.5 (1.0-2.1)	2.4 (1.0-5.5)
Negative likelihood ratio	0.3 (0.1-0.8)	0.6 (0.4-0.9)
Positive predictive value	69 (53-81)	78 (56-92)
Negative predictive value	69 (39-90)	51 (34-68)

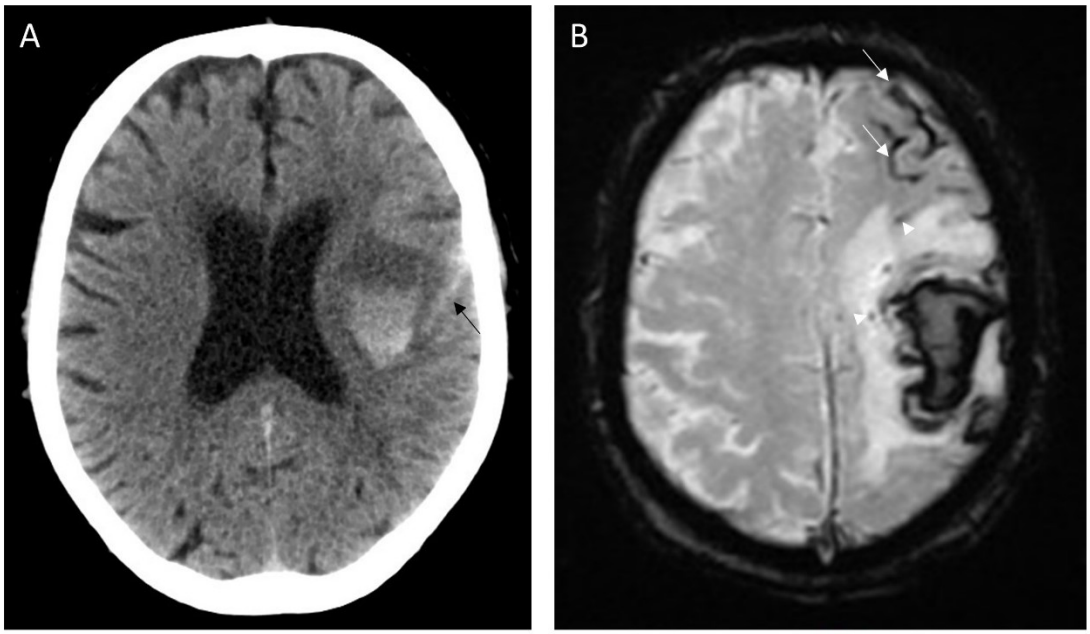
Data are percentage or ratio (95%CI). APOE = apolipoprotein E. CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage.

Examples of true positive and true negative cases are shown in Figure 9.7 and Figure 9.8 respectively. The clinical and imaging findings for the five false positive cases for the rule in criteria and the four false-negative cases for the rule out criteria and are summarised in Figure 9.4 and Figure 9.5 respectively.

Figure 9.7 True positive result for the Edinburgh CT-APOE criteria against the modified Boston criteria

A. Axial non-contrast diagnostic brain CT image showing a left frontal ICH with subarachnoid haemorrhage (black arrow) but no finger-like projections. The APOE genotype is ε2/4. The participant is classified as high risk for CAA on the Edinburgh CT-APOE criteria.

B. Axial T2*-GRE image showing a left frontal ICH with diffuse cortical superficial siderosis (white arrows) and lobar microbleeds (white arrowheads). The participant is classified as probable CAA on the modified Boston criteria.

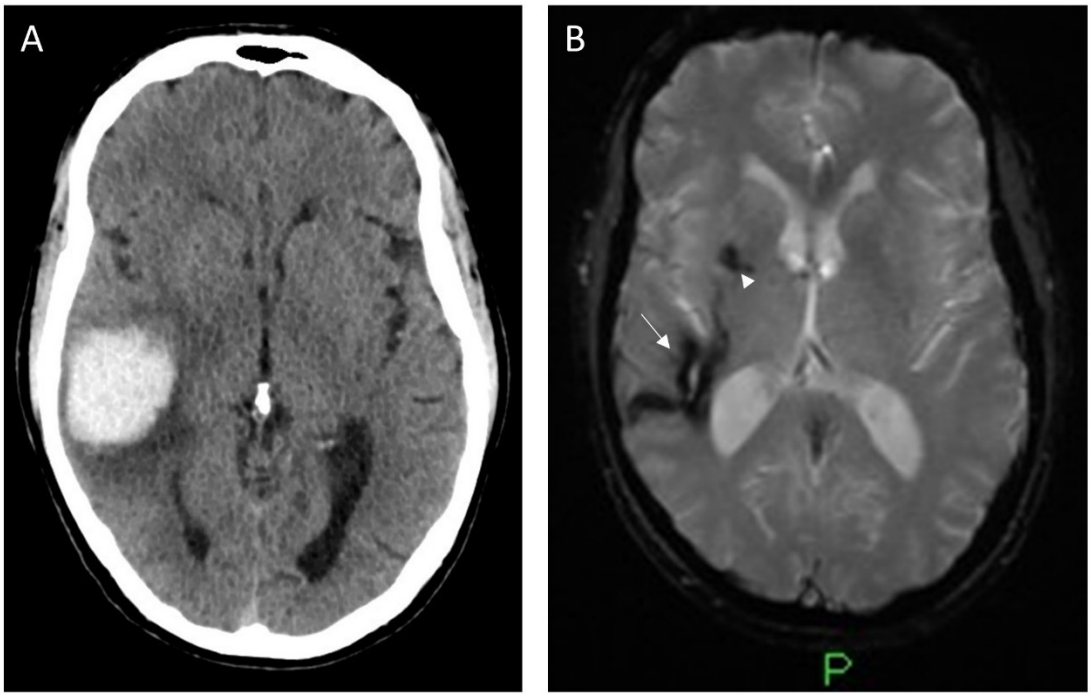


APOE = apolipoprotein. CAA = cerebral amyloid angiopathy. CT = computed tomography. GRE = gradient recalled echo. ICH = intracerebral haemorrhage. MRI = magnetic resonance imaging.

Figure 9.8 True negative result for the Edinburgh CT-APOE criteria against the modified Boston criteria

A. Axial non-contrast diagnostic brain CT image showing a right temporal lobar ICH. There is no subarachnoid haemorrhage or finger-like projections. The APOE genotype is ε3/3.The participant is classified as low risk for CAA on the Edinburgh CT-APOE criteria.

B. Axial T2*-GRE image showing right temporal (white arrow) and basal ganglia (white arrowhead) ICHs. The participant is classified as no CAA on the modified Boston criteria.



APOE = apolipoprotein. CAA = cerebral amyloid angiopathy. CT = computed tomography. GRE = gradient recalled echo. ICH = intracerebral haemorrhage. MRI = magnetic resonance imaging.

9.4.3 Comparison of the Edinburgh and modified Boston criteria against a histopathological reference standard

9.4.3.1 Flow of participants

Nine of the 70 participants with a first-ever lobar ICH diagnosed by non-contrast brain CT and subsequent research brain MRI with T2*-GRE sequence had a research brain autopsy. All nine participants also had APOE genotyping.

9.4.3.2 Comparison of participants with brain CT and MRI who underwent research autopsy versus those who have not had a research autopsy

Comparison of those who had a research autopsy and those did not is difficult due to the small numbers in the research autopsy group. Those underwent autopsy were significantly older than those without an autopsy (Table 9.15). The frequency of subarachnoid haemorrhage and APOE ε4 allele possession between the groups was similar. However, none of the autopsy group had finger-like projections (Table 9.16).

Table 9.15 Baseline clinical features in first-ever lobar ICH participants with a diagnostic non-contrast brain CT scan, APOE genotyping and research brain MRI who had a research brain autopsy (reference standard) versus those who did not

	No research autopsy (n=61)		Research autopsy (n=9)		p value
Age (years); median (IQR)	76	(64-80)	82	(79-83)	0.011
Sex					
Female	35	(57)	7	(78)	
Male	26	(43)	2	(22)	
Co-morbidities					
Hypertension*	31	(51)	4	(44)	1.000
Ischaemic stroke*	2	(3)	0	(0)	1.000
Transient ischaemic attack*	3	(5)	0	(0)	1.000
Dementia*	0	(0)	1	(11)	0.129
Diabetes*	6	(10)	0	(0)	1.000
Atrial fibrillation*	8	(13)	2	(22)	0.607
Myocardial infarction*	2	(3)	1	(11)	0.343
Hyperlipidaemia*	5	(8)	0	(0)	1.000
Smoking status*					
Current	14	(23)	1	(11)	
Ex-smoker	16	(26)	3	(33)	0.804
Never	31	(51)	5	(56)	
Pre-ICH modified Rankin scale; median (IQR)	1	(1-1)	1	(1-2)	0.405
Medications on admission					
Antiplatelet drug(s)*	22	(36)	2	(22)	0.708
Anticoagulant drug(s)*	8	(13)	1	(11)	1.000
Antihypertensive drug(s)*	24	(39)	3	(33)	1.000
Admission GCS; median (IQR)	15	(14-15)	14	(14-15)	0.151
Days between index ICH & CT; median (IQR)	1	(0-2)	1	(0-3)	0.567
Days between CT & MRI; median (IQR)	89	(71-110)	85	(55-112)	0.614

Data are n (%) or median (IQR). * Fisher's exact test. APOE = apolipoprotein E. CAA = cerebral amyloid angiopathy. CT = computed tomography. GCS = Glasgow coma scale. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. MRI = magnetic resonance imaging.

Table 9.16 Non-contrast diagnostic brain CT features and APOE genotype in first-ever lobar ICH participants with a diagnostic non-contrast brain CT scan, APOE genotyping and research brain MRI who had a research brain autopsy (reference standard) versus those who did not

	No research autopsy (n=61)		Research autopsy (n=9)		p value
ICH volume; median (IQR)	19	(9-32)	16	(5-34)	0.498
Intraventricular haemorrhage*	9	(15)	1	(11)	1.000
Subarachnoid haemorrhage*	40	(66)	6	(67)	1.000
Subdural haemorrhage*	9	(15)	0	(0)	0.592
Finger-like projections*	13	(21)	0	(0)	0.193
CT SVD score*					
0	34	(56)	5	(56)	
1	16	(26)	4	(44)	0.336
2	11	(18)	0	(0)	
3	0	(0)	0	(0)	
APOE ε2 allele possession†*	16	(33)	4	(44)	0.704
APOE ε4 allele possession†*	21	(43)	5	(56)	0.717
CT-only CAA category*					
Low	21	(34)	3	(33)	
Intermediate	27	(44)	6	(67)	0.325
High	13	(21)	0	(0)	
CT-APOE CAA category†*					
Low	11	(22)	2	(22)	
Intermediate	19	(39)	3	(33)	1.000
High	19	(39)	4	(44)	

Data are n (%) or median (IQR). * Fisher's exact test. † APOE genotype missing in 12 participants without research autopsy. APOE = apolipoprotein E. CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. MRI = magnetic resonance imaging. SVD = small vessel disease.

9.4.3.3 Comparison of the Edinburgh and modified Boston criteria against the histopathological assessment of CAA

Three of the nine participants had absent or mild parenchymal CAA on histopathological assessment using the consensus Love et al. scale[36] (

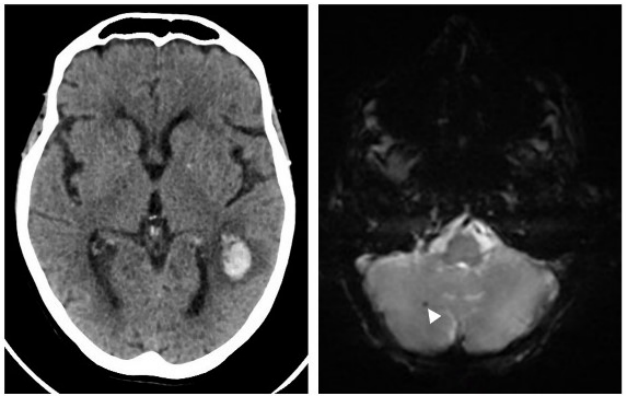
Figure 9.9). Two of these were classified as intermediate risk of CAA on both the CT-only and CT-APOE Edinburgh criteria and the other as low risk on both sets of Edinburgh criteria. All three were classified as probable CAA by the modified Boston criteria.

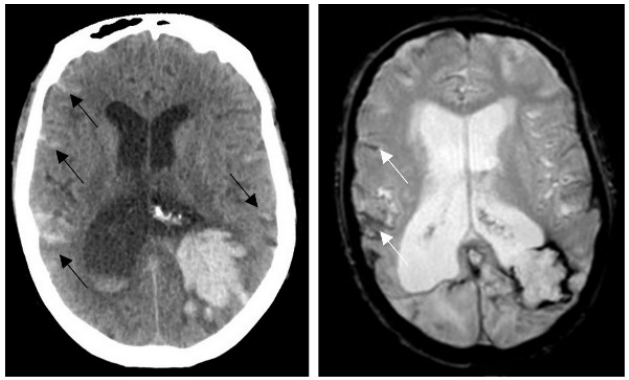
Six of the nine participants had moderate or severe left hemisphere parenchymal CAA on histopathological assessment using the consensus Love et al. scale.[36] Two were classified as low risk and four as intermediate risk using the Edinburgh CT-only criteria. APOE ε4 allele possession upgraded the classification in five, resulting in one low risk, one intermediate risk and four high risk for CAA using the CT-APOE Edinburgh criteria. Four of the participants were classified as probable CAA, one as possible and one as no CAA using the modified Boston criteria.

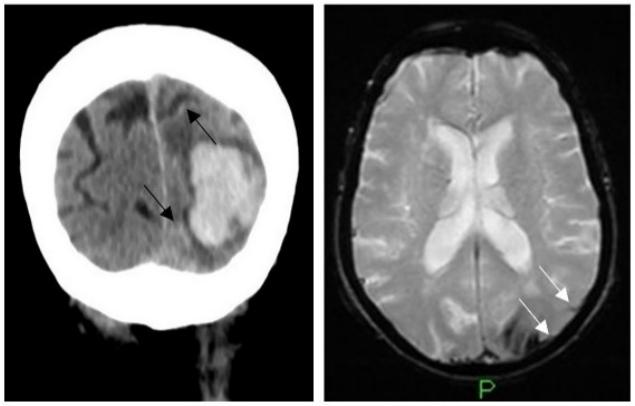
Two participants had a Vonsattel grade <2. One was classified as intermediate risk of CAA on both the CT-only and CT-APOE Edinburgh criteria and the other as low risk on both sets of Edinburgh criteria. Both participants were classified as probable CAA by the modified Boston criteria.

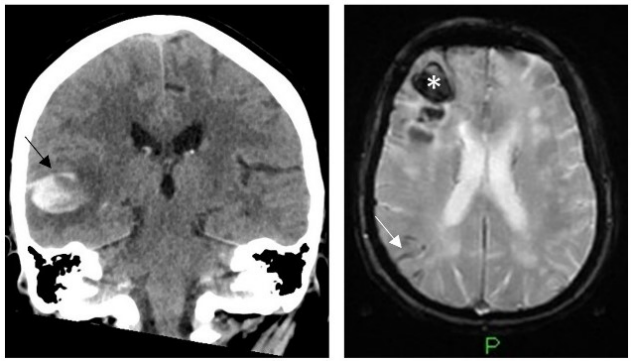
Seven participants had a Vonsattel grade ≥2. Two were classified as low risk and five as intermediate risk using the Edinburgh CT-only criteria. APOE ε4 possession upgraded the classification in five, resulting in one low risk, two intermediate risk and four high risk for CAA using the CT-APOE Edinburgh criteria. Five of the participants were classified as probable CAA, one as possible and one as no CAA using the modified Boston criteria.

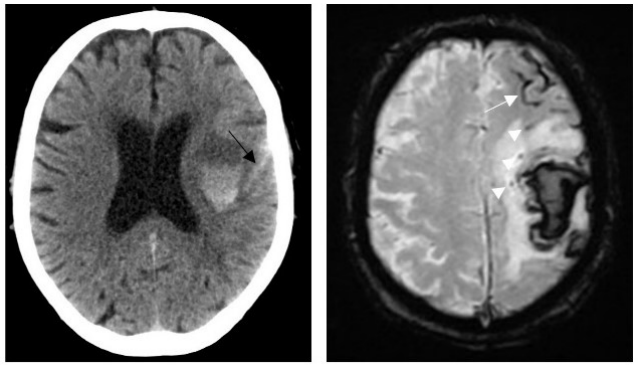
Figure 9.9 Clinical features and non-contrast CT and MRI brain scan features in participants with diagnostic brain CT, research brain MRI, APOE genotyping and research autopsy.

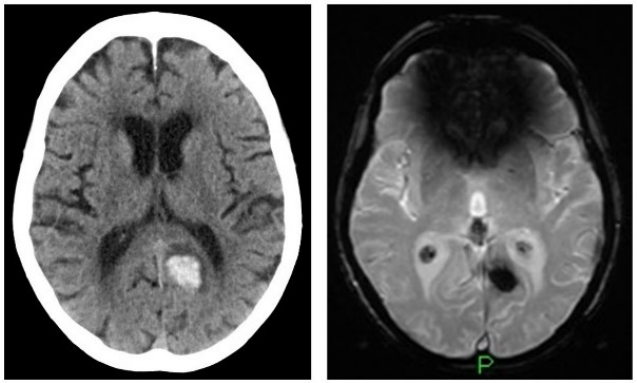
	Clinical details	CT features	MRI features	Example CT and T2*-GRE images	Histopathology features
A	83 year-old female Pre-ICH history of hypertension Pre-ICH antiplatelet drug use APOE genotype ε3/3	Left temporal lobar ICH No subarachnoid haemorrhage No finger-like projections Edinburgh CT-only classification – Low Edinburgh CT-APOE classification - Low	Left temporal lobar ICH No cortical superficial siderosis Cerebellar microbleeds (white arrowhead) Modified Boston criteria – Probable CAA		Absent parenchymal CAA Mild meningeal CAA Vonsattel grade 1 No vasculopathy Severe non-CAA SVD Time from MRI to autopsy = 619 days

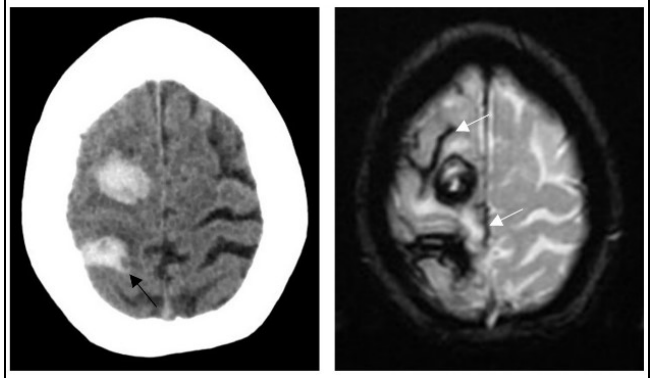
	Clinical details	CT features	MRI features	Example CT and T2*-GRE images	Histopathology features
B	82 year-old female Pre-ICH history of hypertension No pre-ICH antiplatelet or anticoagulant drug use APOE genotype ε3/3	Left temporal lobar ICH Subarachnoid haemorrhage (black arrows) No finger-like projections Edinburgh CT-only classification – Intermediate Edinburgh CT-APOE classification - Intermediate	Two lobar ICHs (left temporal and right occipital lobes) Diffuse cortical superficial siderosis (white arrows) No microbleeds Modified Boston criteria – Probable CAA		Absent parenchymal CAA Absent meningeal CAA Vonsattel grade 0 No vasculopathy Moderate non-CAA SVD Time from MRI to autopsy = 220 days

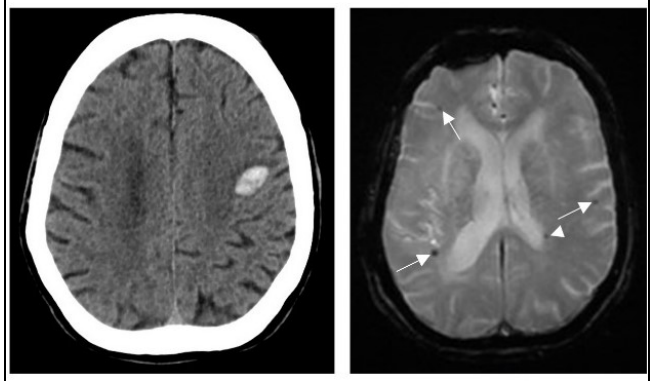
	Clinical details	CT features	MRI features	Example CT and T2*-GRE images	Histopathology features
C	90 year-old female Pre-ICH history of hypertension Pre-ICH anticoagulant drug use (INR 2.3) APOE genotype ε3/3	Left occipital lobar ICH Subarachnoid haemorrhage (black arrow) No finger-like projections Edinburgh CT-only classification – Intermediate Edinburgh CT-APOE classification - Intermediate	Left occipital lobar ICH Focal cortical superficial siderosis (white arrows) No microbleeds Modified Boston criteria – Probable CAA		Mild parenchymal CAA Moderate meningeal CAA Vonsattel grade 2 No vasculopathy Severe non-CAA SVD Time from MRI to autopsy = 519 days

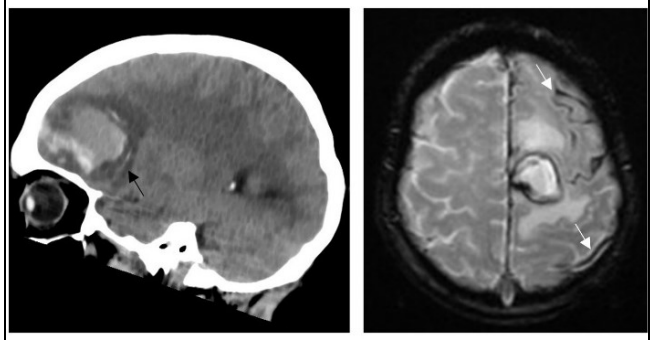
	Clinical details	CT features	MRI features	Example CT and T2*-GRE images	Histopathology features
D	71 year-old female No pre-ICH history of hypertension No pre-ICH antiplatelet or anticoagulant drug use APOE genotype ε2/4	Right temporal lobar ICH Subarachnoid haemorrhage (black arrow) No finger-like projections Edinburgh CT-only classification – Intermediate Edinburgh CT-APOE classification - High	Right temporal and frontal (white asterisk) lobar ICHs Diffuse cortical superficial siderosis (White arrow) No microbleeds Modified Boston criteria – Probable CAA		Moderate parenchymal CAA Severe meningeal CAA Vonsattel grade 3 Vasculopathy present Moderate non-CAA SVD Time from MRI to autopsy = 949 days

	Clinical details	CT features	MRI features	Example CT and T2*-GRE images	Histopathology features
E	79 year-old male Pre-ICH history of hypertension No pre-ICH antiplatelet or anticoagulant drug use APOE genotype ε2/4	Left frontal lobar ICH Subarachnoid haemorrhage (black arrow) No finger-like projections Edinburgh CT-only classification – Intermediate Edinburgh CT-APOE classification - High	Left frontal lobar ICH Diffuse cortical superficial siderosis (white arrow) Multiple strictly lobar microbleeds (white arrowheads) Modified Boston criteria – Probable CAA		Severe parenchymal CAA Severe meningeal CAA Vonsattel grade 3 Vasculopathy present Mild non-CAA SVD Time from MRI to autopsy = 1000 days

	Clinical details	CT features	MRI features	Example CT and T2*-GRE images	Histopathology features
F	88 year-old female No pre-ICH history of hypertension No pre-ICH antiplatelet or anticoagulant drug use APOE genotype ε2/3	Left parietal lobar ICH No subarachnoid haemorrhage No finger-like projections Edinburgh CT-only classification – Low Edinburgh CT-APOE classification - Low	Left parietal lobar ICH No cortical superficial siderosis No microbleeds Modified Boston criteria – Possible CAA		Severe parenchymal CAA Severe meningeal CAA Vonsattel grade 3 Vasculopathy present Severe non-CAA SVD Time from MRI to autopsy = 1994 days

	Clinical details	CT features	MRI features	Example CT and T2*-GRE images	Histopathology features
G	81 year-old female No pre-ICH history of hypertension No pre-ICH antiplatelet or anticoagulant drug use APOE genotype ε3/4	Right frontal and right parietal lobar ICHs Subarachnoid haemorrhage (black arrow) No finger-like projections Edinburgh CT-only classification – Intermediate Edinburgh CT-APOE classification - High	Right frontal and right parietal lobar ICHs Diffuse cortical superficial siderosis (white arrows) No microbleeds Modified Boston criteria – Probable CAA		Severe parenchymal CAA Severe meningeal CAA Vonsattel grade 3 Vasculopathy present Moderate non-CAA SVD Time from MRI to autopsy = 1876 days

	Clinical details	CT features	MRI features	Example CT and T2*-GRE images	Histopathology features
H	83 year-old female No pre-ICH history of hypertension Pre-ICH antiplatelet drug use APOE genotype ε3/4	Left frontal lobar ICH No subarachnoid haemorrhage No finger-like projections Edinburgh CT-only classification – Low Edinburgh CT-APOE classification - Intermediate	Left frontal lobar ICH No cortical superficial siderosis Lobar (white arrows) and deep microbleeds (white arrowhead) Modified Boston criteria – No CAA		Severe parenchymal CAA Severe meningeal CAA Vonsattel grade 3 No vasculopathy Severe non-CAA SVD Time from MRI to autopsy = 321 days

	Clinical details	CT features	MRI features	Example CT and T2*-GRE images	Histopathology features
I	67 year-old male No pre-ICH history of hypertension No pre-ICH antiplatelet or anticoagulant drug use APOE genotype ε2/4	Left frontal lobar ICH Subarachnoid haemorrhage (black arrow) No finger-like projections Edinburgh CT-only classification – Intermediate Edinburgh CT-APOE classification - high	Two left frontal lobar ICHs Diffuse cortical superficial siderosis (white arrows) No microbleeds Modified Boston criteria – Probable CAA		Severe parenchymal CAA Severe meningeal CAA Vonsattel grade 3 Vasculopathy present Mild non-CAA SVD Time from MRI to autopsy = 1712 days

APOE = apolipoprotein. CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. MRI = magnetic resonance imaging. SVD = small vessel diseases.

9.5 Discussion

9.5.1 Main findings

- The Edinburgh CT-only rule out criteria (no subarachnoid haemorrhage or finger-like projections) were 81% sensitive for probable CAA on the modified Boston criteria.
- The Edinburgh CT-only rule in criteria (subarachnoid haemorrhage and finger-like projections) were 96% specific for probable CAA on the modified Boston criteria.
- The Edinburgh CT-APOE rule out criteria (no subarachnoid haemorrhage, APOE ε4 allele possession or finger-like projections) were 89% sensitive for probable CAA on the modified Boston criteria.
- The Edinburgh CT-APOE rule in criteria (subarachnoid haemorrhage plus APOE ε4 allele possession and/or finger-like projections) were 78% specific for probable CAA on the modified Boston criteria.

9.5.2 Strengths of the study

I performed and reported the study accorded to the STARD guidelines for diagnostic accuracy studies.[173] Important strengths are:

- We used prospective case ascertainment to reduce selection bias by inviting all potentially eligible patients to the study.[175, 287]
- I only included participants with first-ever ICH to provide a standard inception point.
- The reference standard was acquired prospectively, and we optimised procedures for the study question.[175]
- The reference standard was offered to all eligible participants, regardless of clinical features or the results of the index test to reduce partial verification bias.[174, 176, 246]
- All included participants underwent the same index test and reference standard to avoid differential verification bias.[174]
- I minimised information bias for the diagnostic non-contrast brain CT index test by standardising imaging format, using the same definition

for CT predictors as I used in the development study, and performing the ratings masked to clinical, genetic and MRI data.[175, 176]

- We minimised information bias for the brain MRI reference standard by performing it at a standardised time point after the index ICH and using a standard MRI acquisition protocol for all participants. I followed published guidance for rating SVD biomarkers on MRI,[77, 107] and performed the ratings masked to clinical, genetic and CT findings.[175, 176]
- I pre-specified the cut-offs for the index tests and reference standard
- I reported the flow of participants through the study and described the differences between those who did and did not undergo the reference standard to illustrate selection bias.[174, 175]
- I described the baseline clinical and radiographic features and the distribution of MRI SVD biomarkers in the study groups to describe the disease spectrum within the included participants.[175, 176]

9.5.3 Limitations of the study

Although I tried to limit selection bias, those undergoing the MRI reference standard were younger, with fewer pre-ICH co-morbidities, less pre-ICH disability and lower CT SVD scores (Table 9.1, Table 9.2, Table 9.8 and Table 9.9). They also had smaller ICH volumes, less frequent intraventricular haemorrhage and had a higher GCS on hospital admission. These differences probably reflect the feasibility of MRI scanning in this patient group and highlight the drawbacks of MRI-based diagnostic criteria in ICH. Importantly, we attempted to offer the MRI reference standard to all eligible participants. Therefore the decision to perform a research MRI was not influenced by clinical characteristics or index test results, thereby limiting partial verification bias. In line with this, the frequencies of subarachnoid haemorrhage, finger-like projections and APOE ε4 allele possession were not significantly different between those who did and did not undergo the reference standard, as were the distribution of classifications using the CT-only and CT-APOE Edinburgh criteria (Table 9.3, Table 9.4, Table 9.10 and Table 9.11).

The sample size in my studies is modest. Seventy participants had both diagnostic non-contrast brain CT and research brain MRI, while 58 had diagnostic non-contrast brain CT, APOE genotyping and research brain MRI. The modest sample sizes result in less precise estimates of diagnostic accuracy.[289]

The ideal assessment of the Edinburgh and modified Boston criteria would be to compare them against histopathological assessment for CAA. Currently, there are only nine LINCHPIN participants with a first-ever lobar ICH, who had diagnostic non-contrast brain CT, research brain MRI and research autopsy, which precludes any conclusions being drawn.

I performed all CT and MRI ratings. Assessment by other raters with different levels of experience is important to assess the diagnostic accuracy of the Edinburgh criteria in a wider setting.

9.5.4 Study findings

The presence of subarachnoid haemorrhage (intermediate and high-risk groups on the Edinburgh CT-only criteria) had good sensitivity (81% (95%CI 66-91)) for probable CAA on the modified Boston criteria. There were eight false negatives, where no subarachnoid haemorrhage was present on the diagnostic CT, but the research MRI showed multiple lobar haemorrhagic foci resulting in a probable CAA classification on the modified Boston criteria (Figure 9.5). There are several possible reasons for this.

The Edinburgh CT-only criteria rely solely on features of the acute haemorrhage on the diagnostic non-contrast brain CT scan. In contrast, the modified Boston criteria include features of previous haemorrhagic lesions, such as cortical superficial siderosis and lobar CMBs, which are not visible on CT. Therefore the modified Boston criteria may have a higher sensitivity for CAA.

The sensitivity of the Edinburgh criteria for CAA-associated lobar ICH is likely to be reduced in certain patient groups. For three of the eight false negative results, the diagnostic CT was performed between 3 and 5 days after the

symptom onset. The sensitivity of the Edinburgh criteria in such situations may be lower than patients scanned acutely as subarachnoid haemorrhage will resolve and the haematoma shape can change with time as the blood products start to resorb.[229] Secondly, the participants included in this study tended to have small ICH volumes (median 18 ml, IQR 7-33) compared with the development study (median 60 ml, IQR 20-118) and the external validation study (median 56 ml, IQR 33-86). In Chapter 8 I showed that the sensitivity of the Edinburgh CT-only criteria was lower in those with smaller ICH volume. All of the eight false negatives had an ICH volume ≤50 ml, and for seven the ICH volume was ≤20 ml.

Finally, the Edinburgh and modified Boston criteria were developed using different definitions of CAA-associated ICH. Therefore some discordance between them is to be expected.

The combination of subarachnoid haemorrhage and finger-like projections (Edinburgh CT-only high-risk group) was extremely specific (96% (95%CI 79-100)) for probable CAA on the modified Boston criteria. There was only one false positive using these Edinburgh CT-only rule in criteria. This occurred in a 78-year-old male who had a single lobar ICH, several lobar CMBs and cortical superficial siderosis, but one thalamic CMB (Figure 9.4). The participant is classified as “no CAA” by the modified Boston criteria given the single deep CMB. However, the relevance of a single deep haemorrhagic lesion in the presence of multiple lobar haemorrhagic lesions on a patient’s likelihood of CAA is unclear,[167] and may indicate coexisting CAA and non-CAA SVDs (Figure 4.13).[229]

The presence of subarachnoid haemorrhage or APOE ε4 allele possession +/- finger-like projections (Edinburgh CT-APOE criteria intermediate and high-risk groups) increased the sensitivity for probable CAA on the modified Boston criteria to 89% (95%CI 72-96). The four false negative results using this cut-off are summarised in Figure 9.5. I have discussed the potential reasons for these above. In particular, three of the four false negatives had an ICH volume ≤5 ml.

The high-risk group on the Edinburgh CT-APOE criteria (subarachnoid haemorrhage plus APOE ε4 allele possession and/or finger-like projections) had a specificity of 78% (95%CI 56-92) for probable CAA on the modified Boston criteria. The five false positive results using this cut-off are summarised in Figure 9.4. Three of these had multiple lobar haemorrhagic lesions but a single deep CMB or old haemorrhage. As discussed above, these participants are classified as “no CAA” by the modified Boston criteria. However, it is unclear whether this is appropriate when there are multiple lobar haemorrhagic lesions given the frequent co-existence of CAA and arteriolosclerosis (Figure 4.13).[167, 229] Another false positive occurred in a 50-year-old female who had a frontal ICH and cortical superficial siderosis. She were classified as “no CAA” on the modified Boston criteria because she was less than 55 years old at the time of her ICH. Whilst CAA is an age-related disease, the validity of a strict age cut-off for diagnosing CAA-associated is uncertain.

9.5.5 Future directions

The Edinburgh CT-only and CT-APOE diagnostic criteria show good sensitivity and specificity for probable CAA on the modified Boston criteria. However the sample size in this study was modest. In particular, the number of participants with diagnostic CT, MRI and histopathology was too small to draw any conclusions.

Future work should aim to assess the diagnostic accuracy of the Edinburgh criteria against the modified Boston criteria in a larger sample to allow more precise estimates. A larger sample size would also allow the diagnostic accuracy of the Edinburgh criteria to be assessed in relevant subgroups, such as those with a small ICH volume and those who have a delay between symptom onset and diagnostic non-contrast brain CT. The inclusion of participants who have been scanned on MRI scanners with different field strengths (1.5T and 3T) and different blood-sensitive sequences (T2*-GRE and SWI) will be important given the effects these factors have on the

sensitivity for detecting CMBs and cortical superficial siderosis.[109, 209, 341-346]

I am currently coordinating a multi-centre diagnostic test accuracy study of the modified Boston criteria and the Edinburgh CT-only and CT-APOE criteria against a histopathological reference standard. This study includes adults with first-ever lobar ICH with a diagnostic non-contrast brain CT, APOE genotype if available, subsequent brain MRI with blood-sensitive sequence and histopathology assessment (cortical biopsy or brain autopsy) for CAA. This is the ideal study design to assess the diagnostic test accuracy of the Edinburgh and modified Boston criteria, and will provide more precise information on the accuracy and concordance of the two sets of criteria.

Another important area for future research is the risk of CAA-associated outcomes, such as recurrent ICH and post-stroke dementia, predicted by the Edinburgh and modified Boston criteria. Studies should assess whether the two sets of diagnostic criteria are additive for future risk of CAA-associated outcomes. For example, are patients classified as low risk of CAA on both the Edinburgh and modified Boston criteria at a lower risk of recurrent ICH than those classified as low risk on only one of the criteria? Conversely, are those classified as high risk by the Edinburgh criteria and probable CAA by the modified Boston criteria at the highest risk of recurrent ICH? What is the significance of discordant classifications by the two diagnostic criteria? Ultimately the question of whether the CT-based Edinburgh criteria can be used to identify patients who will or will not benefit from further assessment of SVD biomarkers using MRI will be important to investigate to inform the rational use of imaging in clinical practice.

Finally, these data highlight some of the drawbacks of the modified Boston criteria. In particular, the presence of a single deep CMB in a patient with multiple lobar haemorrhagic foci results in a “no CAA” classification. However, given the frequent co-existence of CAA and non-CAA SVDs (Figure 4.13), this is likely to lead to misclassification. Rigorous external validation and revision of the modified Boston criteria is required.

Multi-modality imaging assessment of cerebral small vessel disease biomarkers after stroke due to spontaneous intracerebral haemorrhage

Volume 2

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University of Edinburgh

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Section E. Prognostic value of CT SVD biomarkers in SVD-associated ICH

Chapter 10 The association between the Edinburgh diagnostic criteria for CAA-associated lobar ICH and the risk of recurrent ICH

10.1 Introduction

Lobar ICH associated with MRI biomarkers of CAA seems to have a higher risk of recurrent ICH compared with other subtypes of ICH.[105] These biomarkers may also increase the risk of antithrombotic-related ICH.[158] Non-invasive diagnosis of CAA in lobar ICH may therefore be important to estimate prognosis and guide treatment decisions in clinical practice.

The principal approach for non-invasive diagnosis of CAA uses the MRI-based modified Boston criteria.[110] However, many patients with ICH cannot undergo MRI (Figure 3.4) because of contraindications or limited availability. This is particularly the case in low and middle-income countries where over 80% of worldwide deaths due to haemorrhagic stroke occur.[132]

The Edinburgh diagnostic criteria for CAA-associated lobar ICH use two diagnostic CT features (subarachnoid haemorrhage and finger-like projections from the haematoma), along with APOE ε4 genotype if available, to predict the risk of moderate or severe CAA in lobar ICH (Chapter 7)[229] I have shown that in the development setting, the rule-out and rule-in diagnostic criteria showed excellent sensitivity and specificity for moderate or severe CAA respectively (Chapter 7). Furthermore, I have demonstrated that the Edinburgh CT-only criteria has good diagnostic accuracy for CAA in a large multi-centre external validation study (Chapter 8).

The Edinburgh diagnostic criteria may be more widely applicable than the modified Boston criteria given the better availability of CT compared with MRI. However, the association between the Edinburgh diagnostic criteria and the risk of recurrent ICH is unknown.

10.2 Aims

I aimed to investigate the association between the Edinburgh diagnostic criteria for CAA-associated lobar ICH (CT-only and CT and APOE criteria separately) and the risk of recurrent ICH in survivors of lobar ICH.

10.3 Methods

10.3.1 Study design and patients

10.3.1.1 Edinburgh CT-only diagnostic criteria study

I used data from the prospective, community-based LATCH study (section 2.1.3.1). I included consecutive patients living in the NHS Lothian Health Board region who had a spontaneous ICH between 1st June 2010 and 31st May 2013 inclusive, diagnosed on non-contrast brain CT. Some of the LATCH patients were included in the development of the Edinburgh diagnostic criteria.

As an external cohort, I used data from the Clinical Relevance of Microbleeds in Stroke (CROMIS-2) ICH study (ClinicalTrials.gov Identifier: NCT02513316).[347] This was a prospective case-control study of adult patients with and without oral anticoagulant-associated ICH conducted in 79 UK centres. I included participants who had a spontaneous ICH between December 2010 and July 2015 inclusive, diagnosed on non-contrast brain CT (CROMIS-2 cohort).

10.3.1.2 Edinburgh CT and APOE diagnostic criteria study

I used data from the prospective LINCHPIN study (section 2.1.3.2). I included consecutive participants living in the NHS Lothian Health Board region who had a spontaneous ICH between 1st June 2010 and 31st May 2016 inclusive, diagnosed on non-contrast brain CT and had APOE genotyping. Some of the LINCHPIN participants were included in the development of the Edinburgh diagnostic criteria.

As an external cohort, I included participants from the CROMIS-2 study who had a spontaneous ICH between December 2010 and July 2015 inclusive,

diagnosed on non-contrast brain CT and APOE genotyping (CROMIS-2-DNA cohort).

10.3.1.3 Eligibility criteria

I included adult patients (aged ≥16 years) who had a first-ever or recurrent ICH diagnosed by non-contrast brain CT and who survived at least 30 days.

I excluded patients with exclusively extra-axial intracranial haemorrhage, ICH secondary to an underlying cause other than SVDs, and those without a diagnostic quality non-contrast brain CT. I also excluded those who had a recurrent ICH within the first 30 days after the index ICH.

I excluded patients without APOE genotyping from the Edinburgh CT and APOE diagnostic criteria study.

10.3.2 Baseline data collection

Collaborators collected demographics, the date of index ICH symptom onset, the presence of pre-ICH co-morbidities and medication use at the time of ICH and on hospital discharge or at 30 days after ICH by interviewing patients and/or reviewing medical records (Appendix 5).

The co-morbidities of interest were

- Hypertension (defined as either a history of hypertension in medical records before ICH or taking antihypertensive medication at the time of the ICH)
- Dementia (defined as either a diagnosis of dementia in medical records before ICH or if a relative or close friend completed the short form Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) and the score was ≥64[183])
- Prior ischaemic stroke or TIA
- Myocardial infarction
- Atrial fibrillation
- Diabetes mellitus
- Hyperlipidaemia
- Smoking status

The medication use pre-ICH and at hospital discharge or at 30 days after ICH of interest were

- Antiplatelet drug(s)
- Anticoagulant drug(s)
- Anti-hypertensive drug(s)

10.3.3 Image analysis

I reformatted the non-contrast brain CT volume datasets from the LATCH and LINCHPIN cohorts into standard axial, coronal and sagittal planes as described in section 2.2.1.2. A collaborator (Dr David Seiffge) performed the same reformatting of the non-contrast brain CT volume datasets from the CROMIS-2 participants.

I rated the reformatted non-contrast brain CT scans from LATCH and LINCHPIN participants and Dr Seiffge assessed the reformatted non-contrast brain CT scans from CROMIS-2 participants. We assessed the location of the largest ICH,[190] the ICH volume using a modified ABC/2 approach,[191] and the presence or absence extra-axial haemorrhage (subarachnoid, subdural or intraventricular spaces) and finger-like projections arising from the largest haematoma. We calculated the CT SVD burden score[196] based on the presence and severity of white matter lucencies,[193] lacunes[77] and atrophy[195] as described in section 2.2.1.3.

We performed the CT ratings masked to the clinical, genetic and outcome information.

10.3.4 APOE genotyping

Where available, collaborators performed APOE genotyping on DNA extracted from peripheral blood or brain tissue using standard techniques, masked to radiological, clinical and outcome data. I defined APOE $\varepsilon 2$ and APOE $\varepsilon 4$ possession if a participant had at least one $\varepsilon 2$ or $\varepsilon 4$ allele respectively, as described in Chapter 7.[229]

10.3.5 Follow up and outcome

The RUSH team used multiple sources of information to follow-up participants in LATCH and LINCHPIN as described in section 2.1.8.

The CROMIS-2 study team used multiple sources of information to follow-up participants in the CROMIS-2 study for recurrent ICH and death outcomes. The sources of follow-up information used in CROMIS-2 were not identical to those used in LATCH and LINCHPIN, and included postal questionnaires at six months to the participants and their GPs.[347] All outcome events were notified to the central CROMIS-2 study team by each participating hospital research team. The central study team was also notified of all deaths and hospital attendances in study participants from the Health and Social Care Information Centre; this includes data on all admitted patient care episodes, outpatient appointments, and emergency attendances in England.

For this study, I censored follow-up data at three years after the index ICH or on 27th February 2018, whichever occurred first.

I pre-specified the first recurrent ICH as the outcome of interest. I defined recurrent ICH as the onset of new neurological deficits or worsening of pre-existing deficits, anatomically referable to evidence of new ICH on CT or MRI scans.

I pre-specified death as a competing event.

Both the outcome and the competing event were adjudicated by trained members of the RUSH or CROMIS-2 study teams using all available clinical and imaging information, masked to baseline clinical and imaging data.

10.3.6 Sample size

I was unable to calculate the sample size required to assess the association between the Edinburgh diagnostic criteria for CAA-associated lobar ICH and recurrent ICH as no previous studies have assessed this. Instead, I used data from the community-based LATCH and LINCHPIN studies, as well as data from the multi-centre CROMIS-2 study to maximise power.

10.3.7 Missing data

I excluded any cases with missing predictor or outcome variables. I did not impute missing data.

10.3.8 Statistical analysis

I compared the frequency of clinical characteristics, diagnostic non-contrast brain CT features and APOE genotype between the cohorts using χ^2 test (or Fisher's exact test, where appropriate) for categorical variables and the Mann-Whitney U test for non-normally distributed continuous variables.

10.3.8.1 Time to event analyses

I quantified the follow-up time in each cohort using the median and interquartile range, and the completeness of follow-up by calculating the completeness index ([observed follow up/potential follow up]*100).[348]

I performed time to event analyses with the first recurrent ICH as the outcome and treated death as a competing risk. A competing risk is an event which precludes the occurrence of the primary event of interest.[349] Traditional methods for analysing survival data, such as the Kaplan-Meier method and the Cox proportional hazards model assume that competing risks are absent. Their use in the presence of a competing event results in an overestimation of the probability of the outcome and misestimation of the magnitude of relative effects of predictors on the incidence of the event.[349-351] Accounting for competing events is particularly important when the frequency of the competing event is high, as is the case with death after ICH.[350]

Therefore, I used the cumulative incidence function (CIF) to estimate the incidence of recurrent ICH while taking account of the competing risk of death (univariable competing risk analysis). I performed multivariable regression analysis taking into account death as a competing risk using two approaches; the cause-specific hazard function[351, 352] and the subdistribution hazard of the CIF.[353] The cause-specific hazard function[351, 352] represents the instantaneous rate of occurrence of an event among participants who are still event free. The subdistribution hazard

function[353] represents the instantaneous rate of occurrence of the given event type in participants who have not yet experienced that event type.

I performed the analyses in the internal and external cohorts (Edinburgh CT-only study: LATCH and CROMIS-2 cohorts; Edinburgh CT and APOE study: LINCHPIN and CROMIS-2-DNA cohorts) separately before meta-analysis as described below.

Edinburgh CT-only diagnostic criteria study

Primary analysis

I estimated the cumulative incidence function and subdistribution hazard ratio of recurrent ICH in participants with a first-ever ICH as their index event, stratified by ICH location (lobar versus non-lobar) in LATCH and CROMIS-2 separately.

Next, I assessed univariable predictors of recurrent ICH in participants with a first-ever lobar ICH using cumulative incidence and subdistribution hazard ratio, according to sex, Edinburgh CT-only criteria (low versus intermediate versus high; low versus intermediate or high) and CT SVD score (0 versus 1, 2 or 3), separately in the LATCH and CROMIS-2 cohorts. I used Schoenfeld residuals and covariate time interactions to assess whether the variables obeyed the proportional hazard subdistribution assumption.

I then performed a two-step individual participant data meta-analysis[354] of recurrent ICH risk in the LATCH and CROMIS-2 studies. First, I performed competing risks multivariable regression for recurrent ICH using subdistribution and cause-specific hazard models, adjusting for Edinburgh CT-only diagnostic criteria and CT SVD score separately in the LATCH and CROMIS-2 cohorts. Then I synthesised the study level aggregate data using a random effects model with DerSimonian-Laird weights.[355] I used a random effects model due to the differences in study design and baseline characteristics between the LATCH and CROMIS-2 studies.[356, 357]

Secondary analyses

To maximise power, I performed a one-step individual participant data meta-analysis[354] by pooling the LATCH and CROMIS-2 first-ever lobar ICH pooling and generating subdistribution and cause-specific hazard models for

recurrent ICH adjusting for Edinburgh CT-only diagnostic criteria and CT SVD score.

After pooling data from LATCH and CROMIS-2, I calculated the univariable cumulative incidence of recurrent ICH in participants with first-ever and recurrent lobar ICH as their index event, stratified by previous history of ICH, hypertension and dementia, medication on hospital discharge, the individual components of the Edinburgh CT-only diagnostic criteria. I then generated competing risks multivariable regression models for recurrent ICH using subdistribution and cause-specific hazard models; one for the Edinburgh CT-only diagnostic criteria and the second for subarachnoid haemorrhage and finger-like projections (the individual components of the Edinburgh CT-only diagnostic criteria). I adjusted both models for age and sex, history of previous ICH, antihypertensive drug use on discharge and CT SVD score.

Edinburgh CT-APOE diagnostic criteria study

Primary analyses

I assessed univariable predictors of recurrent ICH in participants with a first-ever lobar ICH using the cumulative incidence and subdistribution hazard ratio, according to sex, Edinburgh CT-APOE (low versus intermediate versus high; low versus intermediate or high) and CT SVD (0 versus 1, 2 or 3), separately in the LINCHPIN and CROMIS-2-DNA cohorts. I assessed whether the variables obeyed the proportional hazards assumption using Schoenfeld residuals and assessing covariate time interactions.

I performed competing risks multivariable regression for recurrent ICH using subdistribution and cause-specific hazard models, adjusting for Edinburgh CT-APOE diagnostic criteria and CT SVD score in the CROMIS-2-DNA cohort. I could not perform multivariable regression in the LINCHPIN cohort as the Edinburgh CT-APOE diagnostic criteria did not obey the proportional hazards assumption.

Secondary analyses

I performed a one-step individual participant data meta-analysis[354] by pooling the LINCHPIN and CROMIS-2-DNA first-ever lobar ICH data and

generating subdistribution and cause-specific hazard models for recurrent ICH adjusting for Edinburgh CT-APOE diagnostic criteria and CT SVD score.

I calculated the univariable cumulative incidence of recurrent ICH in participants with first-ever and recurrent lobar ICH as their index event, stratified by APOE ε2 and APOE ε4 allele possession. I then generated competing risks multivariable regression models for recurrent ICH using subdistribution and cause-specific hazard model; one for the Edinburgh CT-APOE diagnostic criteria and the second for subarachnoid haemorrhage, finger-like projections and APOE ε4 allele possession (the individual components of the Edinburgh CT-APOE diagnostic criteria). I adjusted both models for history of previous ICH, antihypertensive drug use on discharge, CT SVD score and APOE ε2 allele possession.

10.3.8.2 Confounders

Blood pressure control and antithrombotic drug use are potential confounders for recurrent ICH.[158, 159] These variables were difficult to treat as time-varying covariates due to the data collection methods in the cohorts. Instead, I took a descriptive approach, quantifying the use of antiplatelet, anticoagulant and antihypertensive drug use at hospital discharge or on day 30 after ICH in key groups (recurrent ICH versus no recurrent ICH; Edinburgh CT-only diagnostic criteria groups; Edinburgh CT-APOE diagnostic criteria groups).

10.4 Results

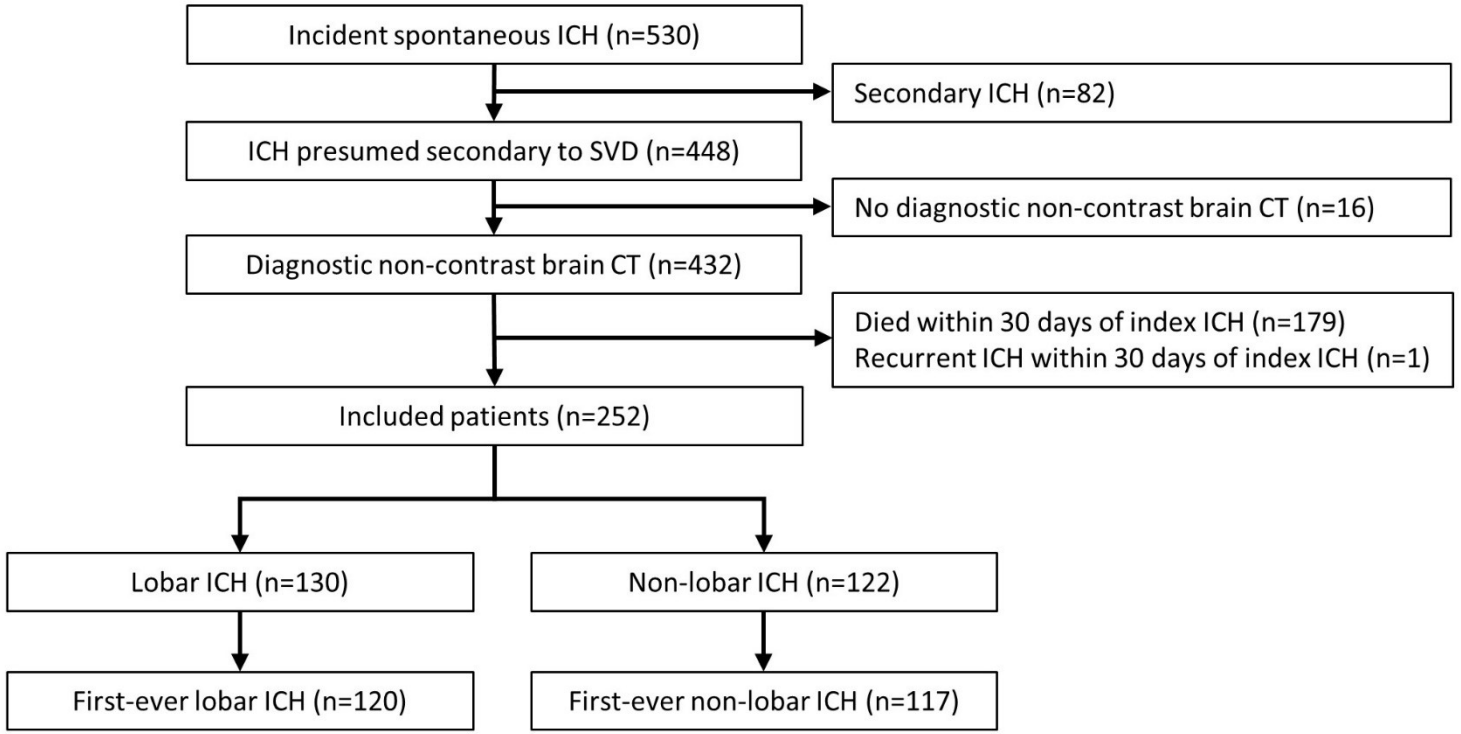
10.4.1 Edinburgh CT-only diagnostic criteria for CAA-associated lobar ICH study

10.4.1.1 Flow of patients

LATCH

There were 448 patients with an ICH presumed secondary to SVDs in the NHS Lothian Health Board region between 1st June 2010 and 31st May 2013 inclusive (Figure 10.1).

Figure 10.1 Flowchart of SVD-associated ICH participants in the LATCH study inclusive who had a diagnostic non-contrast brain CT and survived at least 30 days.



CT = computed tomography. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. SVD = small vessel disease.

Four hundred and thirty-two had diagnostic non-contrast brain CT, of whom 252 survived at least 30 days without having a recurrent ICH.

CROMIS-2

There were 1026 participants with an ICH presumed secondary to SVDs in the CROMIS-2 study between 8th December 2010 and 28th July 2015 (Figure 10.2). Five had missing data for the outcome, while 70 died or had a recurrent ICH within 30 days of their index event. Therefore, I included 951 participants, of whom 372 had a lobar ICH.

10.4.1.2 Comparison of LATCH and CROMIS-2 participants with first-ever SVD-associated lobar ICH

Participants with first-ever lobar ICH in LATCH and CROMIS-2 were of similar ages and had similar ICH volumes and frequency of finger-like projections (Table 10.1 and Table 10.2). CROMIS-2 had a significantly higher proportion of male participants. The frequency of pre-ICH diabetes, atrial fibrillation and hyperlipidaemia was higher in CROMIS-2. There was more frequent pre-ICH use of anticoagulant and antihypertensive drugs in CROMIS-2 but less frequent pre-ICH antiplatelet drug use. Subarachnoid haemorrhage and CT measures of SVDs were more frequent in LATCH, while more CROMIS-2 participants were classified as low risk by the Edinburgh CT-only diagnostic criteria. A higher proportion of LATCH participants had a recurrent ICH or died during follow up.

10.4.1.3 Completeness of follow up

The median duration of follow-up in LATCH was 1095 days (IQR 324-1095), with 99.8% completeness of follow-up. The median duration of follow-up in CROMIS-2 was 1094 days (IQR 687-1094), with 95.8% completeness of follow-up.

10.4.1.4 Cumulative incidence of recurrent ICH in the presence of death as a competing event.

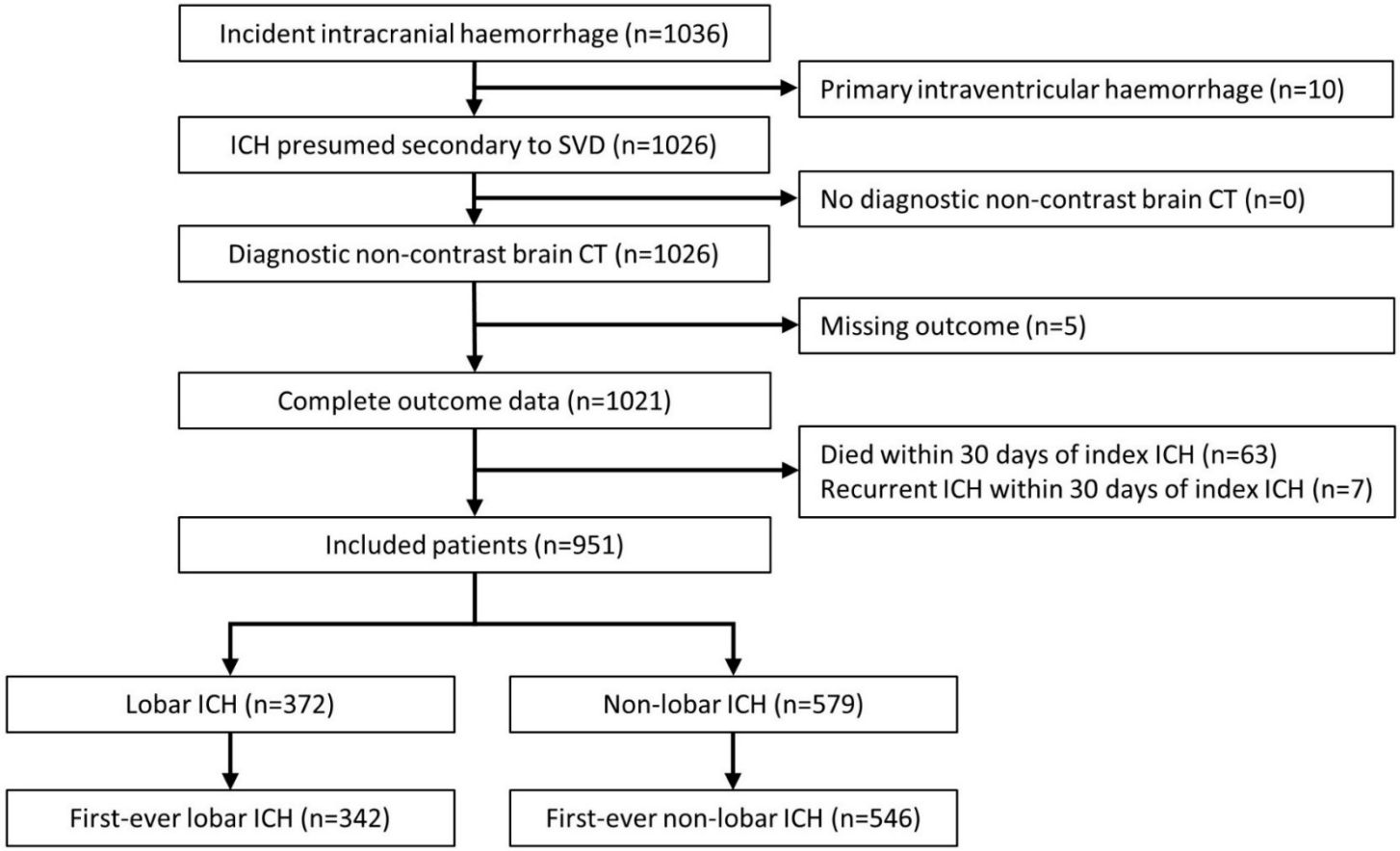
The cumulative incidences of recurrent ICH and death in LATCH and CROMIS-2 participants with first-ever ICH and first-ever lobar ICH are shown in Figure 10.3. The cumulative incidence of death far exceeded that of

recurrent ICH at all time points in all cohorts, indicating that death is a substantial competing event for recurrent ICH.

10.4.1.5 Risk of recurrent ICH stratified by ICH location in participants with first-ever SVD-associated ICH

One hundred and seventeen (49%) LATCH participants had a first-ever non-lobar ICH, 5 of whom suffered a recurrent ICH during follow up (3-year cumulative incidence rate 4.3%, 95%CI 1.6-9.1). One hundred and twenty (51%) LATCH participants had a first-ever lobar ICH, 17 of whom had a recurrent ICH during follow up (3-year cumulative incidence rate 14.2%, 95%CI 8.6-21.1, p=0.009, Figure 10.4). The 3 year cumulative incidence of death in first-ever non-lobar ICH (38.5%, 95%CI 29.6-47.2) was similar to first-ever lobar ICH (38.5%, 95%CI 29.7-47.1, p=0.861, Figure 10.4).

Figure 10.2 Flowchart of SVD-associated ICH participants in the CROMIS-2 study who had a diagnostic non-contrast brain CT and survived at least 30 days.



CROMIS = clinical relevance of microbleeds in stroke. CT = computed tomography. ICH = intracerebral haemorrhage. SVD = small vessel disease.

Table 10.1 Baseline clinical and outcome in first-ever SVD-associated lobar ICH participants in the LATCH and CROMIS-2 cohorts

	LATCH first-ever lobar ICH (n=120)	CROMIS-2 first-ever lobar ICH (n=342)	p value
Age (years); median (IQR)	78 (70-82)	76 (68-83)	0.608
Unknown	*0*	*3*	
Sex			
Female	73 (61)	157 (46)	0.005
Male	47 (39)	185 (54)	
Co-morbidities			
Hypertension	66 (55)	214 (64)	0.093
Unknown	*0*	*6*	
Ischaemic stroke	12 (10)	42 (12)	0.497
Unknown	*0*	*1*	
Transient ischaemic attack	9 (8)	43 (13)	0.105
Unknown	*0*	*12*	
Dementia	19 (16)	33 (10)	0.074
Unknown	*0*	*5*	
Diabetes	12 (10)	68 (20)	0.013
Unknown	*0*	*3*	
Atrial fibrillation	25 (21)	120 (38)	0.001
Unknown	*0*	*27*	
Myocardial infarction	9 (8)	29 (9)	0.716
Unknown	*0*	*3*	
Hyperlipidaemia	17 (14)	161 (49)	<0.001
Unknown	*0*	*12*	
Smoking status			
Current	25 (24)	25 (8)	<0.001
Ex-smoker	34 (32)	131 (40)	
Never	47 (44)	173 (53)	
Unknown	*14*	*13*	
Medications on admission			
Antiplatelet drug(s)	54 (45)	90 (26)	<0.001
Unknown	*0*	*1*	
Anticoagulant drug(s)	13 (11)	141 (42)	<0.001
Unknown	*0*	*2*	
Antihypertensive drug(s)	50 (42)	203 (60)	<0.001
Unknown	*0*	*2*	
Admission GCS; median (IQR)	14 (13-15)	15 (14-15)	<0.001
Unknown	*0*	*5*	
Medications on discharge			
Antiplatelet drug(s)*	3 (3)	16 (5)	0.425
Died during admission	*6*	*?*	
Unknown	*0*	*15*	
Anticoagulant drug(s)*	4 (4)	12 (4)	1.000
Died during admission	*6*	*?*	
Unknown	*0*	*16*	
Antihypertensive drug(s)	51 (45)	230 (71)	<0.001
Died during admission	*6*	*?*	
Unknown	*0*	*16*	
Outcome			
Recurrent ICH	17 (14)	25 (7)	<0.001
Death	46 (38)	65 (19)	
Censored	57 (48)	252 (74)	

Data are n (%) or median (IQR). * = Fisher's exact test. GCS = Glasgow coma scale. CROMIS = clinical relevance of microbleeds in stroke. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. SVD = small vessel disease.

Table 10.2 Non-contrast CT brain features in first-ever SVD-associated lobar ICH participants in the LATCH and CROMIS-2 cohorts

	LATCH first-ever lobar ICH (n=120)	CROMIS-2 first-ever lobar ICH (n=342)	p value
Multiple acute ICH	10 (8)	5 (2)	<0.001
Location of largest ICH*			
Frontal	50 (42)	51 (15)	
Parietal	35 (29)	160 (47)	
Temporal	21 (18)	30 (9)	<0.001
Occipital	14 (12)	97 (28)	
Insular	0 (0)	4 (1)	
ICH volume (ml); median (IQR)	17 (6-38)	15 (5-29)	0.173
Intraventricular haemorrhage	28 (23)	44 (13)	0.007
Subarachnoid haemorrhage	78 (65)	132 (39)	<0.001
Subdural haemorrhage	15 (13)	6 (2)	<0.001
Finger-like projections	20 (17)	78 (23)	0.157
Number of lacunes; median (IQR)	0 (0-0)	0 (0-0)	<0.001
Anterior white matter lucencies			
0	25 (21)	217 (64)	
1	68 (57)	76 (22)	<0.001
2	27 (23)	49 (14)	
Posterior white matter lucencies			
0	42 (35)	208 (61)	
1	30 (25)	48 (14)	<0.001
2	48 (40)	86 (25)	
Central atrophy			
0	31 (26)	47 (14)	
1	79 (66)	240 (71)	0.003
2	10 (8)	55 (16)	
Cortical atrophy			
0	28 (23)	99 (29)	
1	63 (53)	183 (54)	0.217
2	29 (24)	60 (18)	
CT SVD score*			
0	50 (42)	194 (57)	
1	40 (33)	103 (30)	0.001
2	26 (22)	44 (13)	
3	4 (3)	1 (0)	
CT SVD score 1, 2 or 3	70 (58)	148 (43)	0.004
Edinburgh CT-only CAA criteria			
Low	42 (35)	177 (52)	
Intermediate	58 (48)	120 (35)	0.006
High	20 (17)	45 (13)	
Edinburgh CT-only CAA criteria Intermediate/high	78 (65)	165 (48)	0.002

Data are n (%) or median (IQR). * = Fisher's exact test. CAA = cerebral amyloid angiopathy. CROMIS = clinical relevance of microbleeds in stroke. CT = computed tomography. GCS = Glasgow coma scale. ICH = intracerebral haemorrhage. . LATCH = Lothian audit of the treatment of cerebral haemorrhage. SVD = small vessel disease.

Figure 10.3 Cumulative incidence of recurrent ICH and death during follow up.

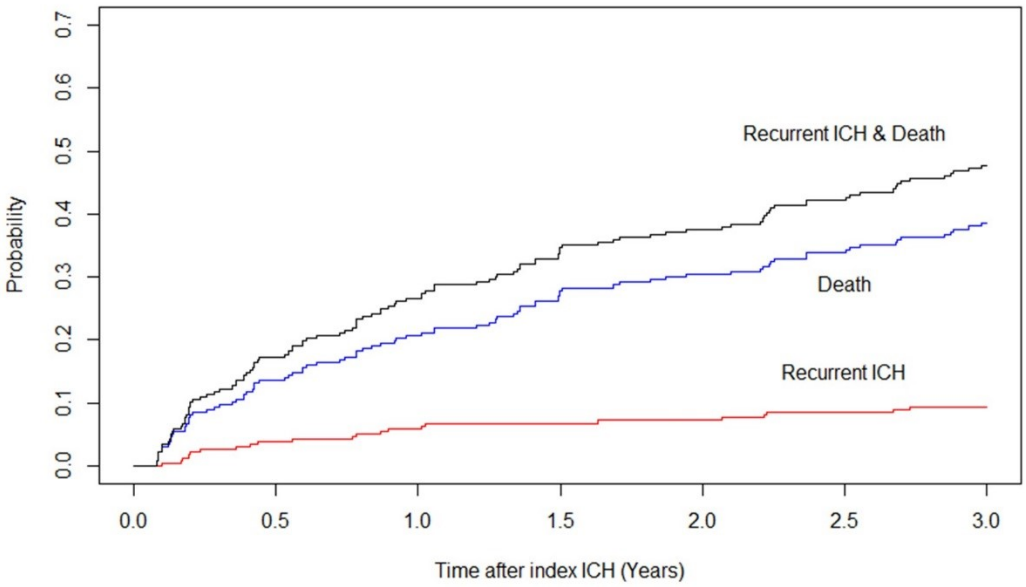
A. First-ever SVD-associated ICH in LATCH

B. First-ever SVD-associated ICH in CROMIS-2

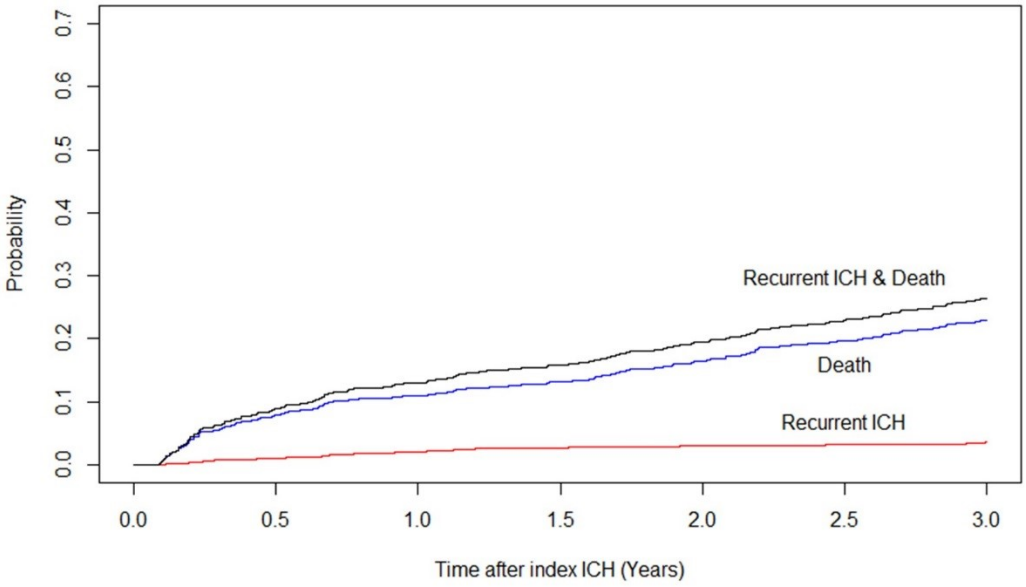
C. First-ever SVD-associated lobar ICH in LATCH

D. First-ever SVD-associated lobar ICH in CROMIS-2

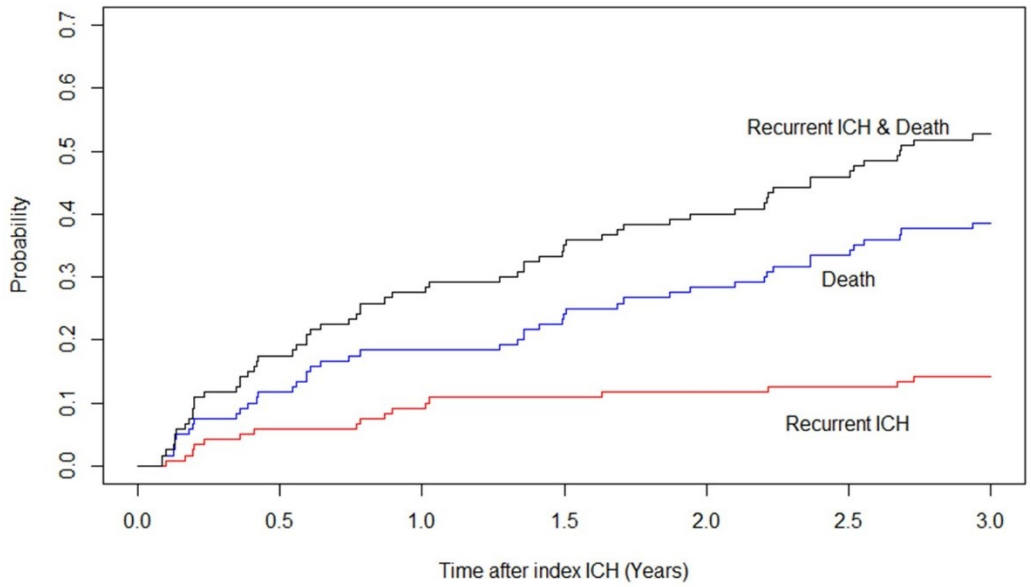
A



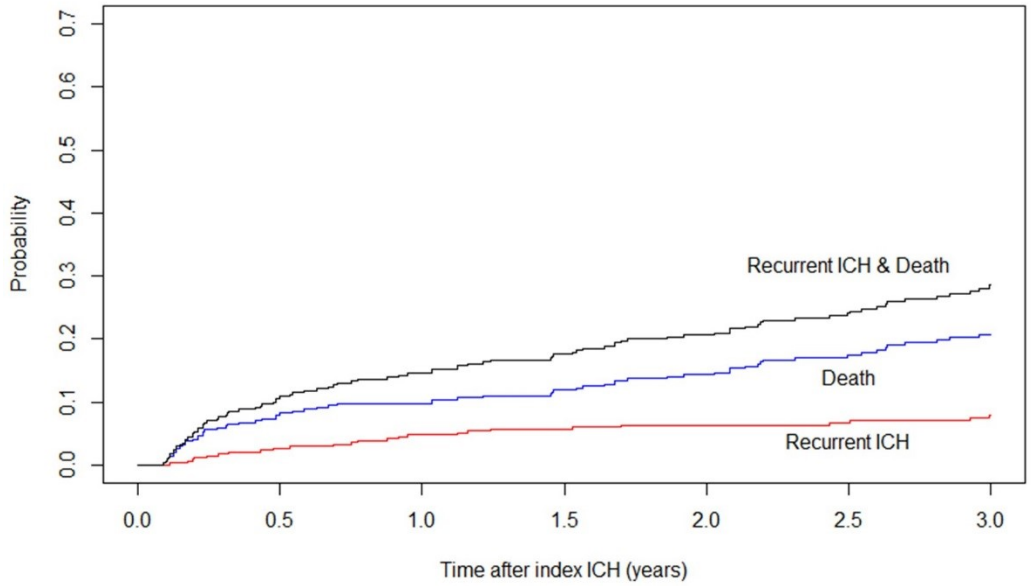
B



C

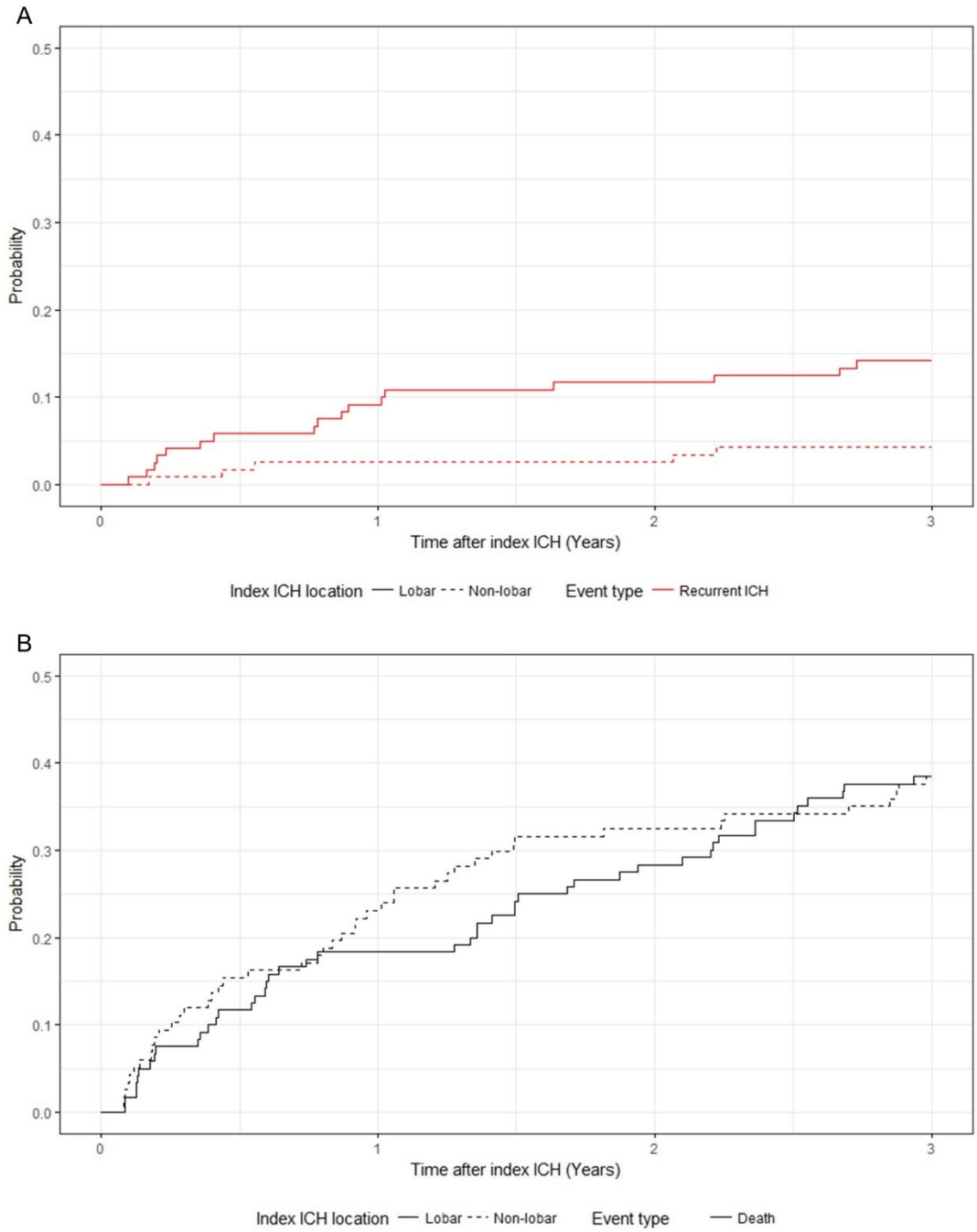


D



CROMIS = clinical relevance of microbleeds in stroke. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. SVD= small vessel disease.

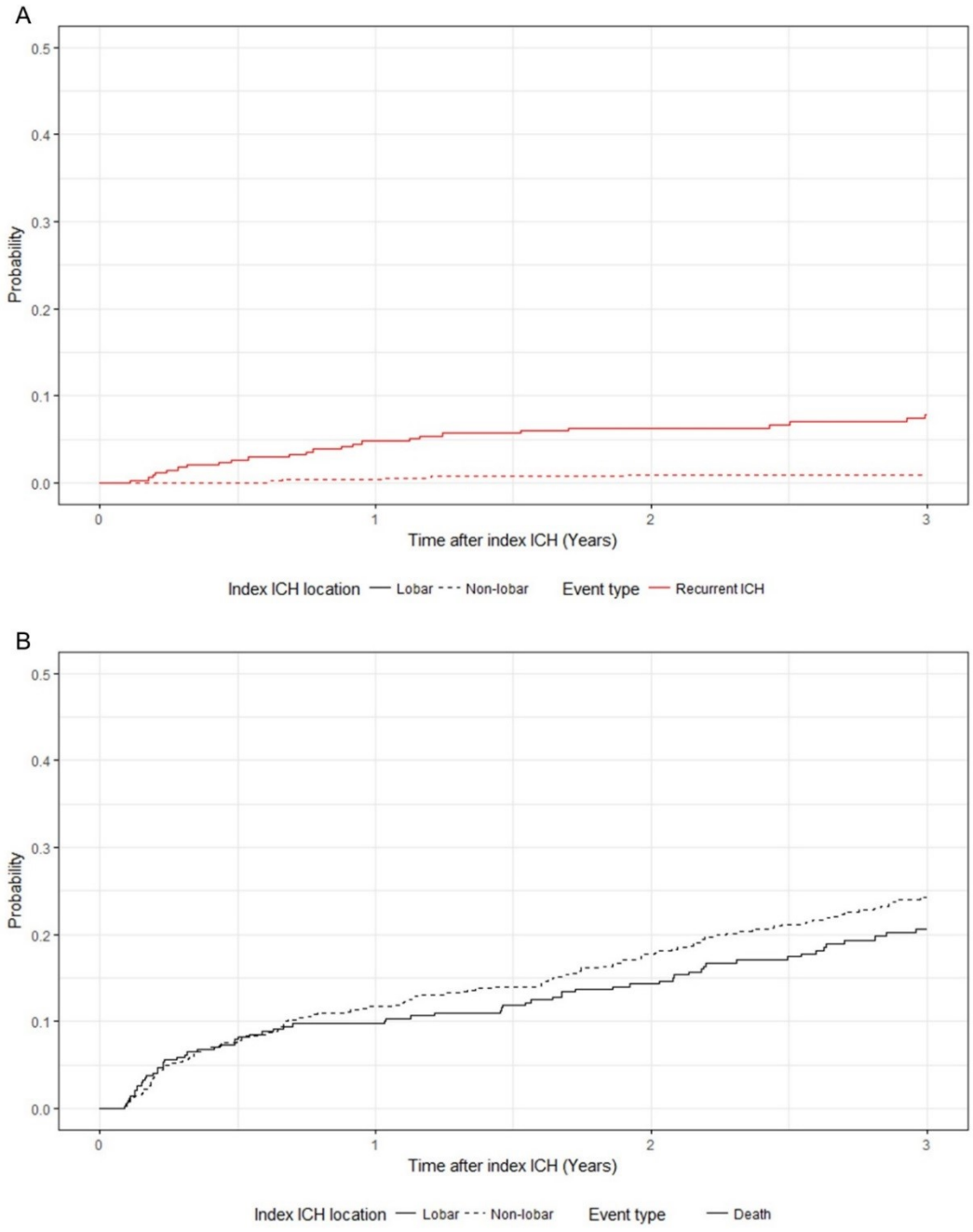
Figure 10.4 Cumulative incidence of A. Recurrent ICH and B. Death in first-ever SVD-associated ICH in LATCH according to the index ICH location



ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. SVD = small vessel disease.

Five hundred and forty-six (61%) CROMIS-2 participants had a first-ever non-lobar ICH, 5 of whom suffered a recurrent ICH during follow up (3-year

Figure 10.5 Cumulative incidence of A. Recurrent ICH and B. Death in first-ever SVD-associated ICH in CROMIS-2 according to the index ICH location



CROMIS = clinical relevance of microbleeds in stroke. ICH = intracerebral haemorrhage. SVD = small vessel disease.

cumulative incidence rate 1.0%, 95%CI 0.4-2.1). 342 (39%) CROMIS-2 participants had a first-ever lobar ICH, 25 of whom had a recurrent ICH during follow up (3-year cumulative incidence rate 7.9%, 95%CI 5.2-11.1,

p<0.001, Figure 10.5). The 3 year cumulative incidence of death in first-ever non-lobar ICH (24.2%, 95%CI 20.6-28.0) was similar to first-ever lobar ICH (20.6%, 95%CI 16.3-25.3, p=0.244, Figure 10.5).

In both LATCH and CROMIS-2, a lobar ICH location significantly increased both the relative incidence (subdistribution hazard ratio) and the rate (cause-specific hazard ratio) of recurrent ICH, with no significant effect on the relative incidence and rate of death (Table 10.3).

Given the low incidence of recurrent ICH in non-lobar ICH, I focused my further analyses on participants with first-ever lobar ICH.

10.4.1.6 Risk of recurrent ICH in participants with first-ever SVD-associated lobar ICH

LATCH cohort

Seventeen of the 120 LATCH participants with first-ever SVD-associated lobar ICH had a recurrent ICH, and 46 died during follow up.

The 3 year cumulative incidence rate for recurrent ICH and death according to sex, CT SVD score and Edinburgh CT-only diagnostic criteria are shown in Table 10.4 and Figure 10.6.

The 3 year cumulative incidence rate for recurrent ICH generally increased with the Edinburgh CT-only CAA criteria (low risk 4.8%, 95%CI 0.8-14.4); intermediate risk 17.2%, 95%CI 8.8-28.1; high risk 25.0%, 95%CI 8.6-45.6, p=0.054). Those classified as intermediate or high risk on the Edinburgh CT-only criteria (19.3%, 95%CI 11.4-28.8) had a significantly higher 3 year cumulative incidence rate for recurrent ICH compared with low risk (4.8%, 95%CI 0.8-14.4, p=0.029). The 3 year cumulative incidence of death was similar across the Edinburgh CT-only risk categories (Figure 10.6).

There was no significant change in the 3 year cumulative incidence of recurrent ICH with the CT SVD score. The cumulative incidence of death was higher in those with a CT SVD score of 1, 2 or 3 versus CT SVD score 0, although this did not reach statistical significance (Table 10.4 and Figure 10.6).

The univariable subdistribution and cause-specific hazard models for sex, CT SVD score and Edinburgh CT-only diagnostic criteria are shown in Table 10.5. High risk and intermediate or high risk on the Edinburgh CT-only criteria significantly increased both the relative incidence (subdistribution hazard ratio) and the rate (cause-specific hazard ratio) of recurrent ICH relative to the low-risk category, with no significant effect on the relative incidence and rate of death. The CT SVD score had no significant effect on the subdistribution hazard ratio or the cause-specific hazard ratio of recurrent ICH. Those with a CT SVD score of 1, 2 or 3 tended to have an increased hazard of death. However, this did not reach statistical significance.

In multivariable models adjusting for CT SVD score and Edinburgh CT-only criteria (Table 10.6), high risk on the Edinburgh CT-only criteria was associated with an increased risk (subdistribution hazard ratio 6.38, 95%CI 1.23-33.3) and rate (cause-specific hazard ratio 7.78, 95%CI 1.51-40.2) of recurrent ICH relative to the low risk category.

Table 10.3 Sub-distribution and cause-specific hazard models for recurrent ICH and death in LATCH and CROMIS-2 participants with first-ever SVD-associated ICH according to index ICH location

	Sub-distribution hazard model				Cause-specific hazard model			
	Recurrent ICH		Death		Recurrent ICH		Death	
	Hazard ratio	p value	Hazard ratio	p value	Hazard ratio	p value	Hazard ratio	p value
LATCH								
Non-lobar ICH	1.00 (Reference)	--	1.00 (Reference)	--	1.00 (Reference)	--	1.00 (Reference)	--
Lobar ICH	3.50 (1.30-9.45)	0.013	0.96 (0.64-1.45)	0.859	3.49 (1.29-9.45)	0.014	1.04 (0.69-1.58)	0.838
CROMIS-2								
Non-lobar ICH	1.00 (Reference)	--	1.00 (Reference)	--	1.00 (Reference)	--	1.00 (Reference)	--
Lobar ICH	8.33 (3.19-21.74)	<0.001	0.84 (0.62-1.13)	0.243	8.27 (3.17-21.60)	<0.001	0.87 (0.65-1.18)	0.372

Data are hazard ratio (95%CI) or p values. CROMIS = clinical relevance of microbleeds in stroke. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. SVD= small vessel disease.

Table 10.4 Three year cumulative incidence of recurrent ICH and death in 120 LATCH participants with first-ever SVD-associated lobar ICH

	Number of participants	Recurrent ICH			Death		
		Number of events	3 year cumulative incidence rate	p value	Number of events	3 year cumulative incidence rate	p value
Sex							
Female	73	11	15.1% (8.0-24.3)	0.730	26	35.6% (24.8-46.6)	0.406
Male	47	6	12.9% (5.2-24.4)		20	43.0% (28.4-56.8)	
CT SVD score							
0	50	7	14.0% (6.1-25.2)	0.909	16	32.0% (19.6-45.1)	0.172
1, 2, 3	70	10	14.4% (7.3-23.7)		30	43.1% (31.2-54.4)	
Edinburgh CT-only CAA criteria							
Low	42	2	4.8% (0.8-14.4)	0.054	18	42.9% (27.6-57.3)	0.646
Intermediate	58	10	17.2% (8.8-28.1)		20	34.5% (22.5-46.8)	
High	20	5	25.0% (8.6-45.6)		8	40.7% (18.6-61.9)	
Edinburgh CT-only CAA criteria							
Low	42	2	4.8% (0.8-14.4)	0.045	18	42.9% (27.6-57.3)	0.541
Intermediate/ High	78	15	19.3% (11.4-28.8		28	36.1% (25.5-46.8)	

Data are% (95%CI). CAA – cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. SVD = small vessel disease.

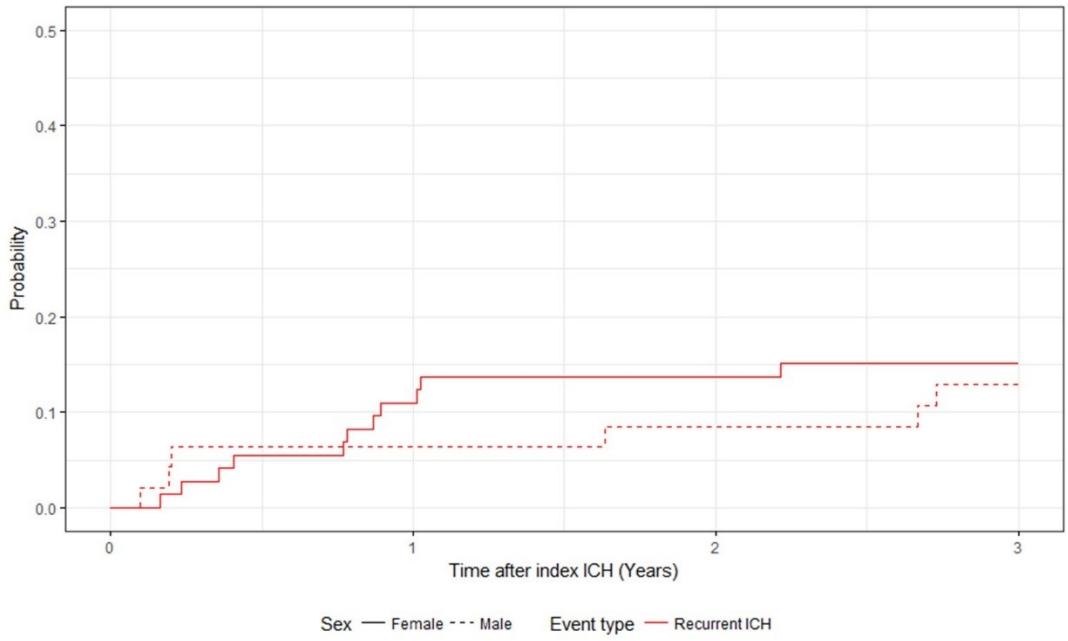
Figure 10.6 Cumulative incidence of recurrent ICH and death in first-ever SVD-associated lobar ICH in LATCH

A & B. Recurrent ICH and death stratified by sex

C & D Recurrent ICH and death stratified by CT SVD score

E to H Recurrent ICH and death stratified by the Edinburgh CT-only CAA criteria

A



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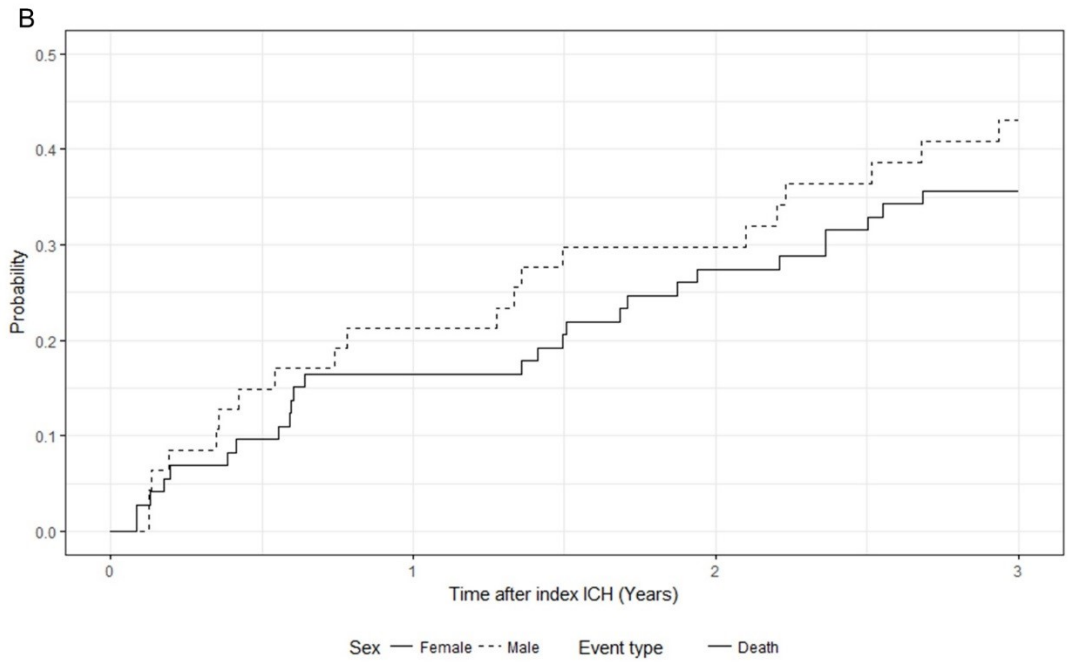

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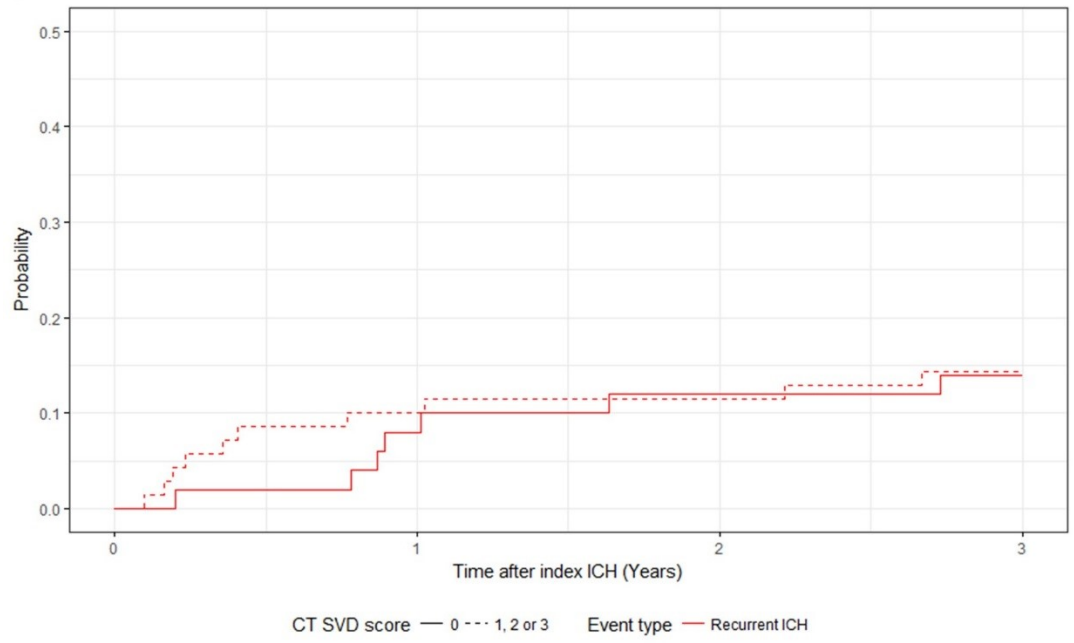
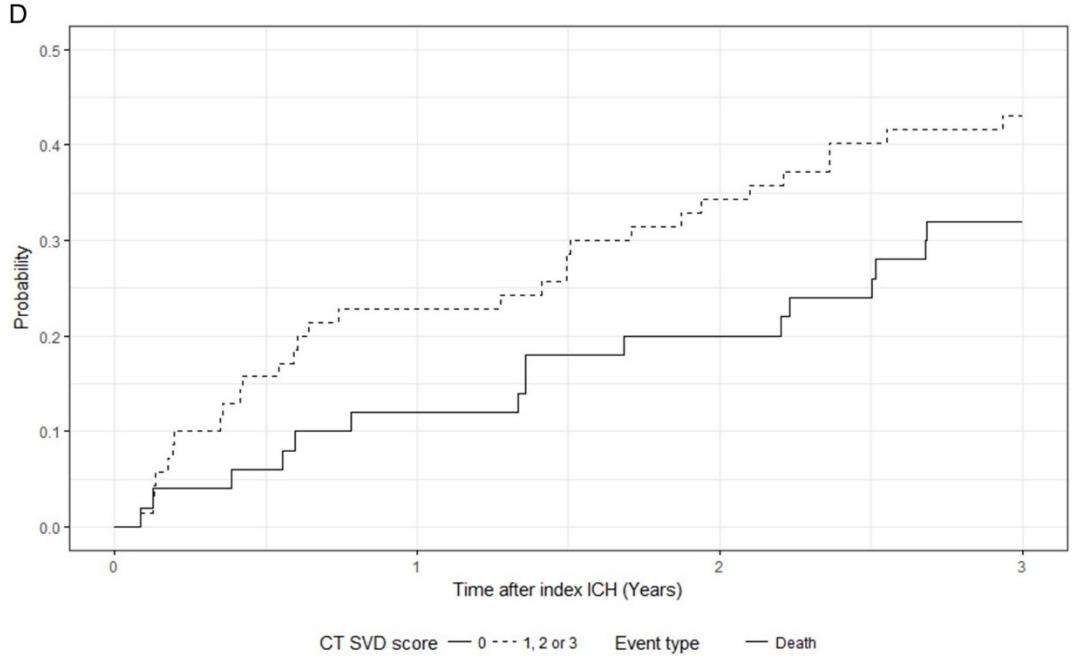
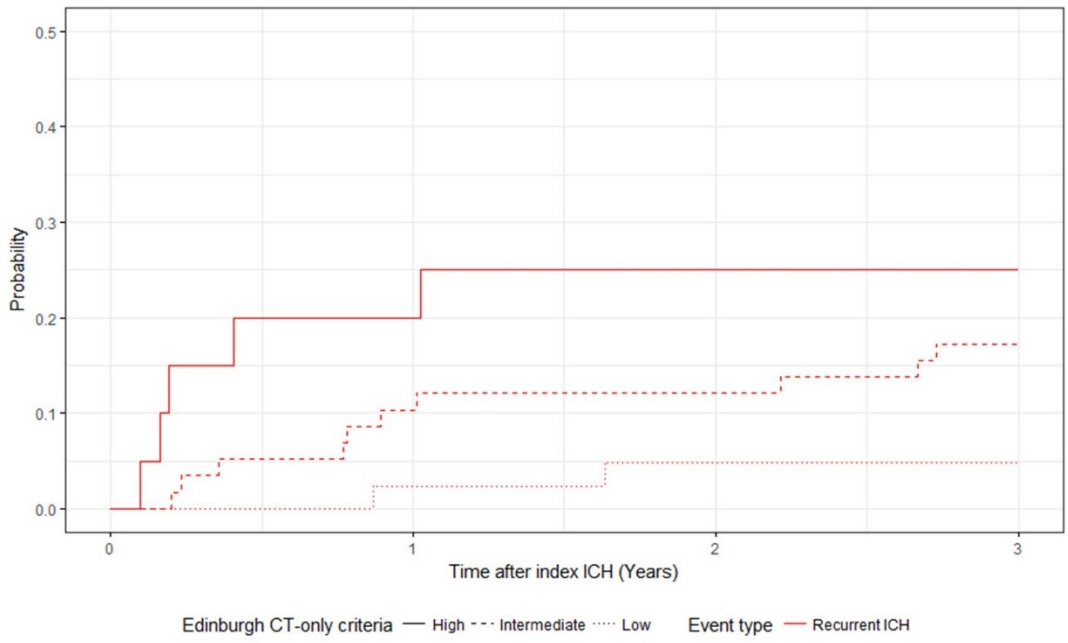

0.5
0.4
0.3
0.2
0.1
0.0
Probability
0
1
2
3
Time after index ICH (Years)
CT SVD score
0
1, 2 or 3
Event type
Recurrent ICH

D


0.5
0.4
0.3
0.2
0.1
0.0
Probability
0
1
2
3
Time after index ICH (Years)
CT SVD score
0
1, 2 or 3
Event type
Death

E


0.5
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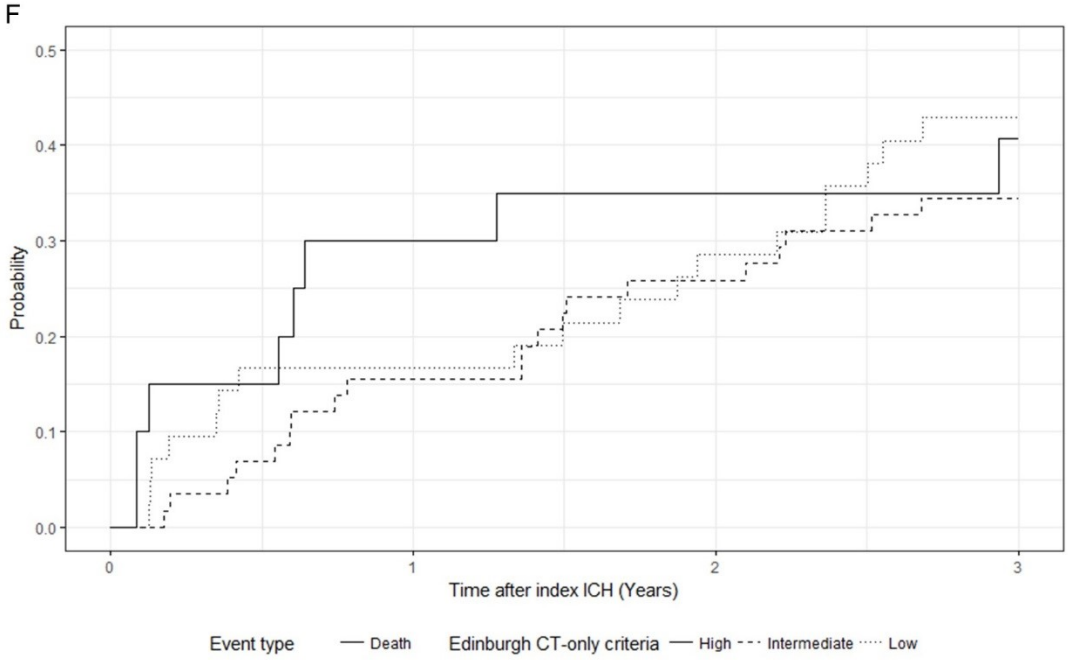
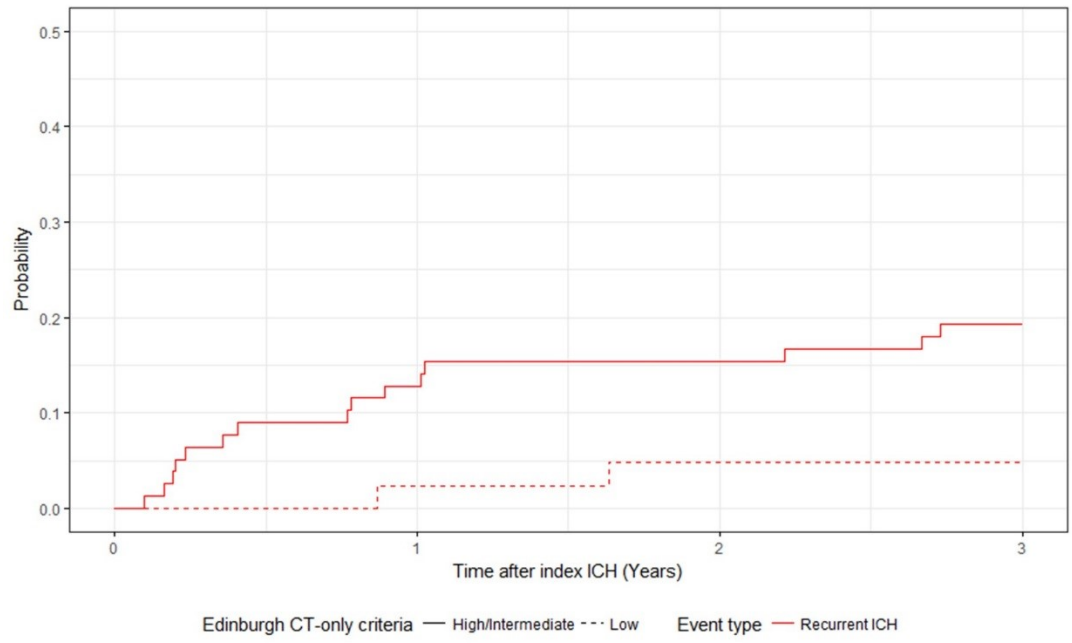
Probability
0
1
2
3
Time after index ICH (Years)
Edinburgh CT-only criteria
High
Intermediate
Low
Event type
Recurrent ICH

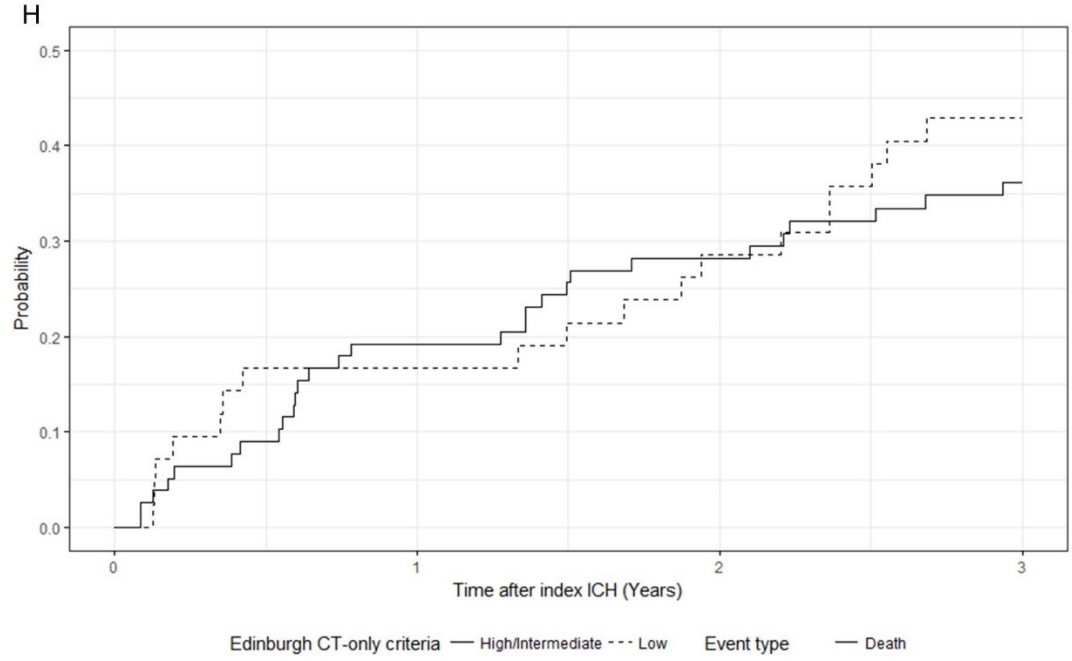
F


0.5
0.4
0.3
0.2
0.1
0.0
Probability
0
1
2
3
Time after index ICH (Years)
Event type
Death
Edinburgh CT-only criteria
High
Intermediate
Low

G



H



CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. CROMIS = clinical relevance of microbleeds in stroke. SVD = small vessel disease.

Table 10.5 Univariable sub-distribution and cause-specific hazard models for recurrent ICH and death in 120 LATCH participants with first-ever SVD-associated lobar ICH

	Sub-distribution hazard model						Cause-specific hazard model					
	Recurrent ICH			Death			Recurrent ICH			Death		
	Hazard ratio		p value	Hazard ratio		p value	Hazard ratio		p value	Hazard ratio		p value
Sex												
Female	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	-	1.00	(Reference)	--
Male	0.84	(0.31-2.25)	0.726	1.28	(0.72-2.28)	0.404	0.88	(0.32-2.37)	0.792	1.24	(0.69-2.22)	0.475
CT SVD score												
0	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	-	1.00	(Reference)	--
1, 2, 3	1.06	(0.41-2.74)	0.910	1.52	(0.84-2.75)	0.170	1.21	(0.46-3.18)	0.699	1.60	(0.87-2.94)	0.129
Edinburgh CT-only CAA criteria												
Low	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Intermediate	3.84	(0.86-17.2)	0.078	0.77	(0.41-1.43)	0.410	3.73	(0.82-17.0)	0.090	0.84	(0.44-1.59)	0.594
High	6.36	(1.24-32.8)	0.027	1.03	(0.44-2.45)	0.939	7.70	(1.49-39.8)	0.015	1.43	(0.62-3.29)	0.405
Edinburgh CT-only CAA criteria												
Low	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Intermediate/high	4.42	(1.03-19.0)	0.045	0.83	(0.46-1.49)	0.532	4.50	(1.03-19.7)	0.046	0.95	(0.53-1.72)	0.870

Data are hazard ratio (95%CI). CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. SVD = small vessel disease.

Table 10.6 Multivariable sub-distribution and cause-specific hazard models for recurrent ICH and death in 120 LATCH participants with first-ever SVD-associated lobar ICH

	Sub-distribution hazard model						Cause-specific hazard model					
	Recurrent ICH			Death			Recurrent ICH			Death		
	Hazard ratio		p value	Hazard ratio		p value	Hazard ratio		p value	Hazard ratio		p value
CT SVD score												
0	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
1, 2, 3	1.15	(0.43-3.04)	0.780	1.46	(0.79-2.70)	0.223	1.32	(0.50-3.52)	0.574	1.57	(0.85-2.90)	0.151
Edinburgh CT-only CAA criteria												
Low	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Intermediate	3.92	(0.82-18.8)	0.087	0.81	(0.43-1.53)	0.524	3.91	(0.85-18.0)	0.080	0.91	(0.48-1.73)	0.769
High	6.38	(1.23-33.3)	0.028	1.01	(0.42-2.39)	0.990	7.78	(1.51-40.2)	0.014	1.47	(0.64-3.40)	0.365

Data are hazard ratio (95%CI). CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. SVD = small vessel disease.

CROMIS-2 cohort

Twenty-five of the 342 CROMIS-2 participants with first-ever SVD-associated lobar ICH had a recurrent ICH, and 65 died during follow up.

The 3 year cumulative incidence rate for recurrent ICH and death according to sex, CT SVD score and Edinburgh CT-only diagnostic criteria are shown in Table 10.7 and Figure 10.7.

The 3 year cumulative incidence rate for recurrent ICH was significantly higher in those with CT SVD score of 1, 2 or 3 compared with a CT SVD of 0 (11.4%, 95%CI 6.8-17.4 versus 5.2%, 95%CI 2.5-9.2, p=0.030). The 3 year cumulative incidence of death was also significantly higher in those with a CT SVD score of 1, 2 or 3. There was no significant change in the 3 year cumulative incidence of recurrent ICH with the Edinburgh CT-only diagnostic criteria, although the point estimate for high risk on the Edinburgh CT-only diagnostic criteria was nearly double the low and intermediate risk groups.

The univariable subdistribution and cause-specific hazard models for sex, CT SVD score and Edinburgh CT-only diagnostic criteria are shown in Table 10.8. CT SVD score 1, 2 or 3 significantly increased both the relative incidence (subdistribution hazard ratio) and rate (cause-specific hazard ratio) of recurrent ICH and of death. The Edinburgh CT-only criteria had no significant effect on the subdistribution hazard ratio or cause-specific hazard ratio of recurrent ICH.

In multivariable models adjusting for CT SVD score and Edinburgh CT-only criteria (Table 10.9), CT SVD score of 1, 2 or 3 was associated with an increased risk (subdistribution hazard ratio 2.37, 95%CI 1.05-5.35) and rate (cause-specific hazard ratio 2.49, 95%CI 1.10-5.64) of recurrent ICH. The Edinburgh CT-only criteria had no significant effect on the subdistribution hazard ratio or cause-specific hazard ratio of recurrent ICH.

Table 10.7 Three year cumulative incidence of recurrent ICH and death in 342 CROMIS-2 participants with first-ever SVD-associated lobar ICH

	Number of participants	Recurrent ICH			Death		
		Number of events	3 year cumulative incidence rate	p value	Number of events	3 year cumulative incidence rate	p value
Sex							
Female	157	14	9.6% (5.5-15.1)	0.262	35	25.2% (18.2-32.9)	0.150
Male	185	11	6.4% (3.4-10.8)		30	16.9% (11.8-22.8)	
CT SVD score							
0	194	9	5.2% (2.5-9.2)	0.030	27	14.6% (9.9-20.2)	0.009
1, 2, 3	148	16	11.4% (6.8-17.4)		38	28.4% (20.9-36.4)	
Edinburgh CT-only CAA criteria							
Low	177	11	6.9% (3.6-11.5)	0.277	29	18.2% (12.5-24.6)	0.398
Intermediate	120	8	7.1% (3.2-12.9)		27	24.0% (16.4-32.3)	
High	45	6	13.6% (5.4-25.6)		9	10.6% (10.1-33.7)	
Edinburgh CT-only CAA criteria							
Low	177	11	6.9% (3.6-11.5)	0.434	29	18.2% (12.5-24.6)	0.217
Intermediate/high	165	14	9.0% (5.1-14.1)		36	23.2% (16.8-30.2)	

Data are % (95%CI). CAA = cerebral amyloid angiopathy. CROMIS = clinical relevance of microbleeds in stroke. CT = computed tomography. ICH = intracerebral haemorrhage. SVD = small vessel disease.

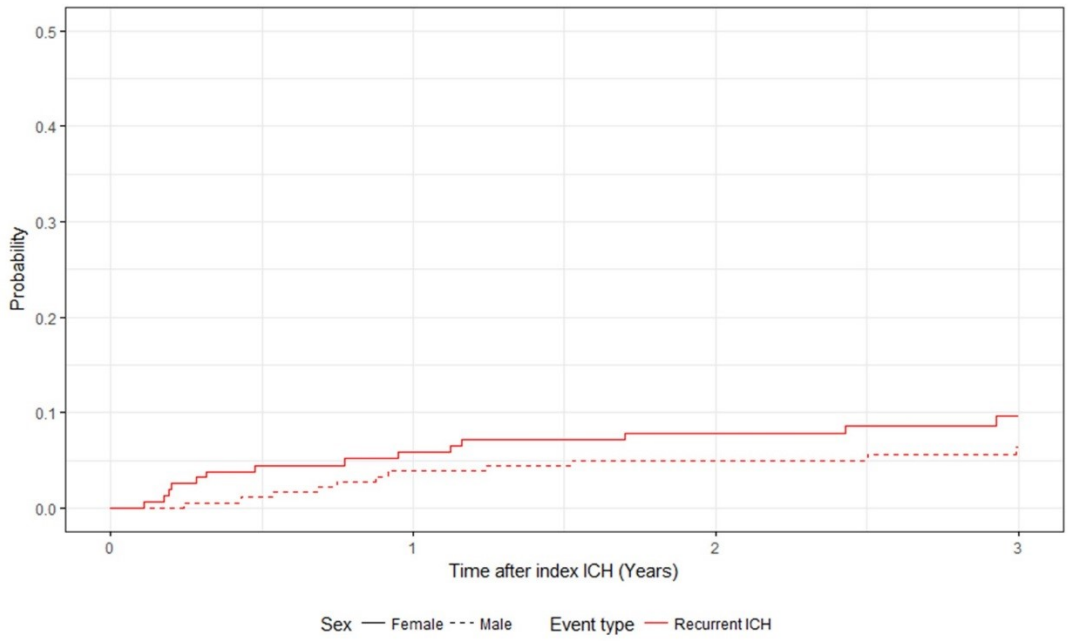
Figure 10.7 Cumulative incidence of recurrent ICH and death in first-ever SVD-associated lobar ICH in CROMIS-2

A & B. Recurrent ICH and death stratified by sex

C & D Recurrent ICH and death stratified by CT SVD score

E to H Recurrent ICH and death stratified by the Edinburgh CT-only CAA criteria

A



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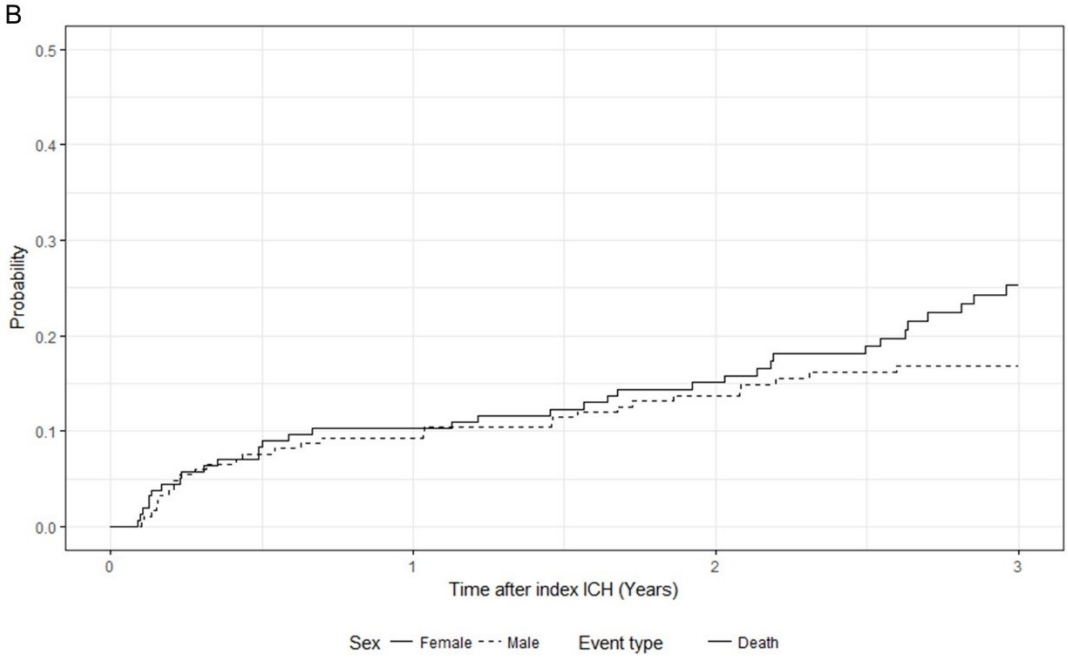

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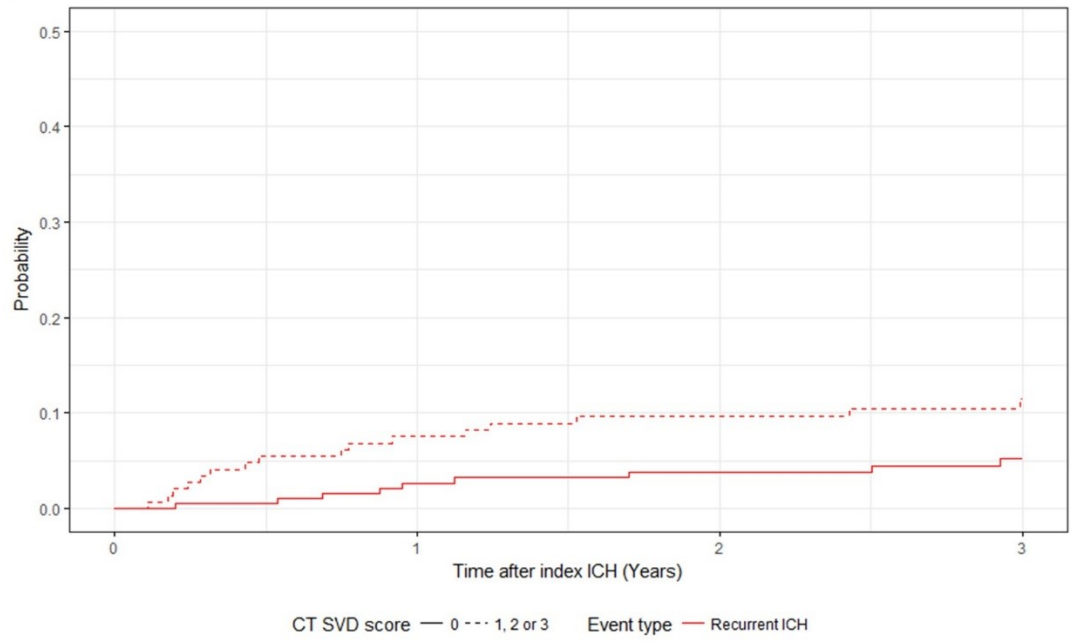
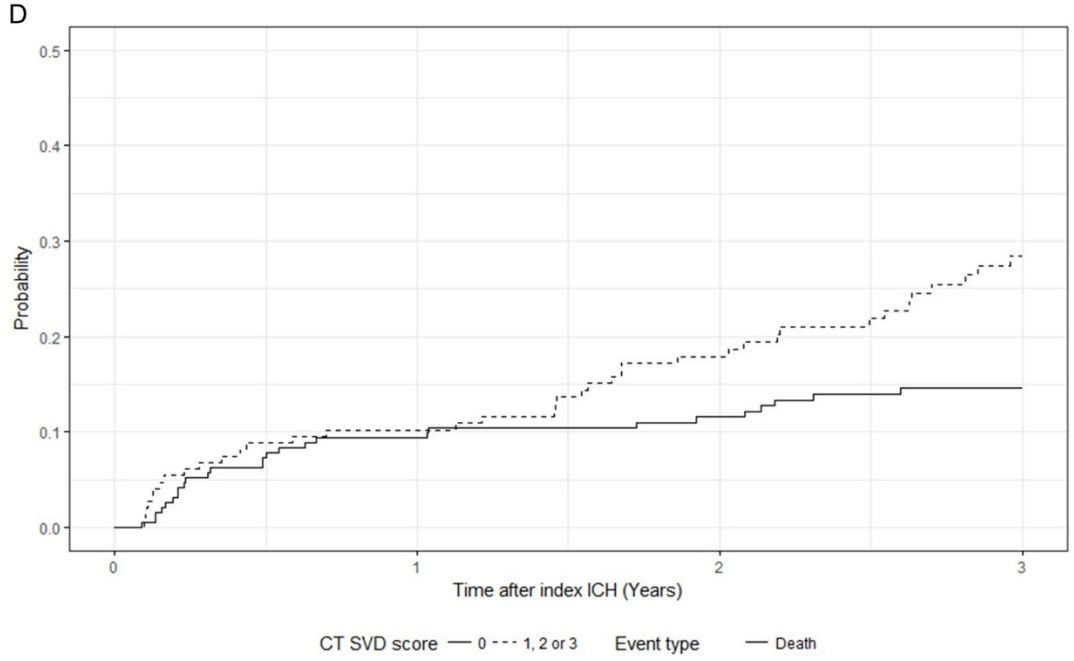
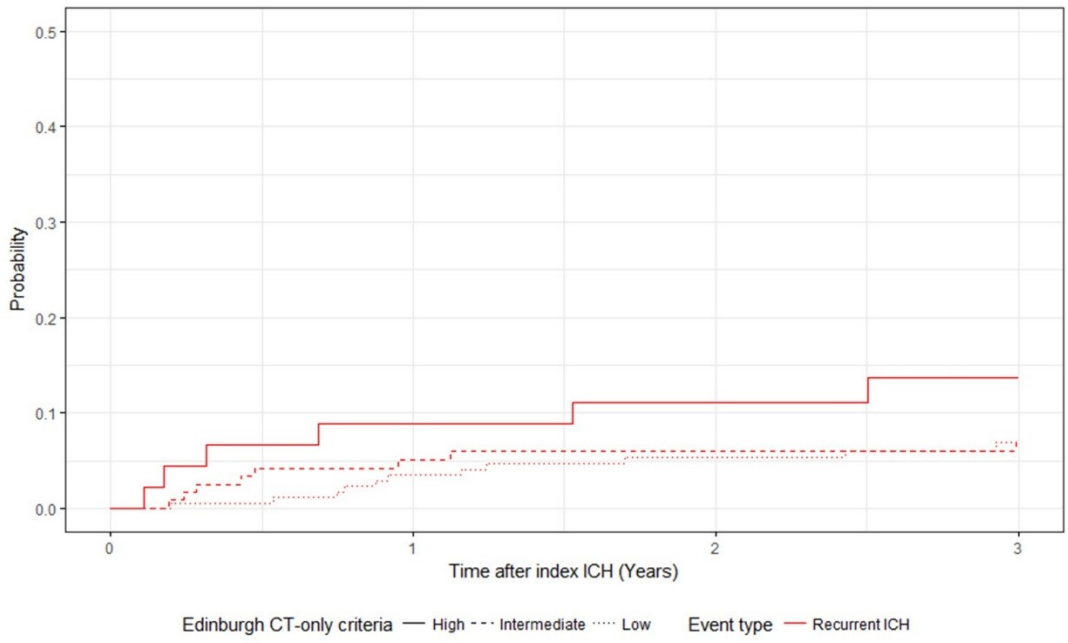

0.5
0.4
0.3
0.2
0.1
0.0
Probability
0
1
2
3
Time after index ICH (Years)
CT SVD score
0
1, 2 or 3
Event type
Recurrent ICH

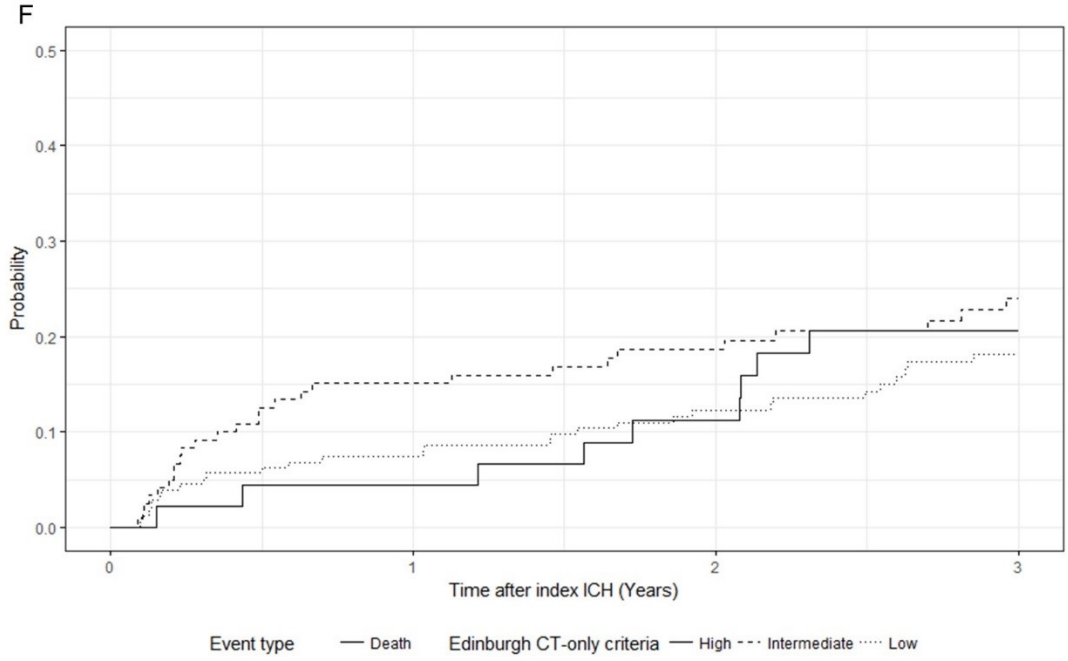
D


0.5
0.4
0.3
0.2
0.1
0.0
Probability
0
1
2
3
Time after index ICH (Years)
CT SVD score
0
1, 2 or 3
Event type
Death

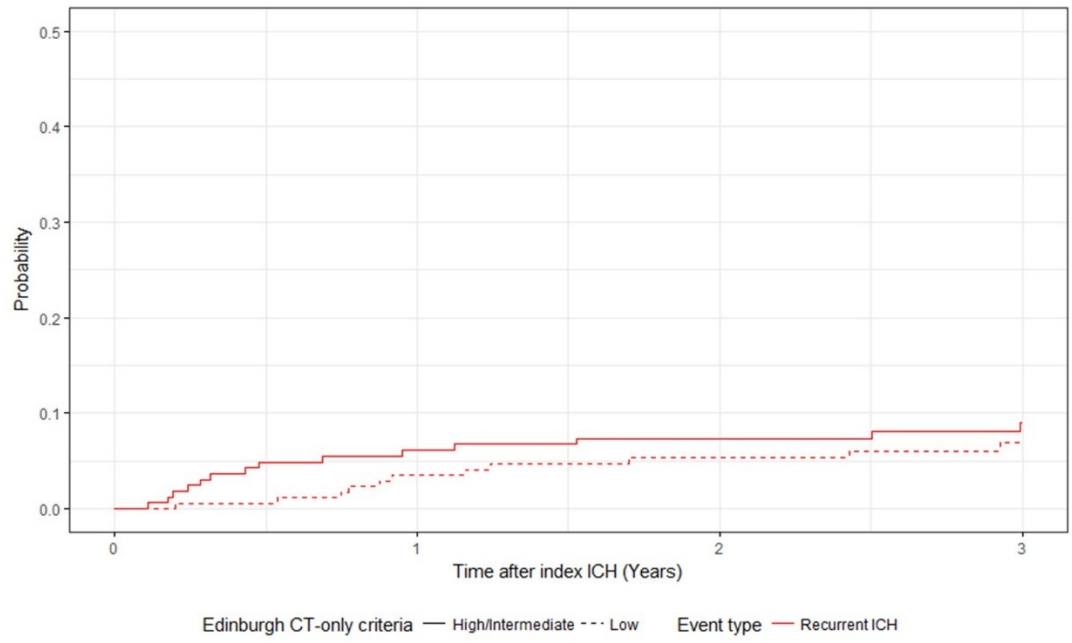
E


0.5
0.4
0.3
0.2
0.1
0.0
Probability
0
1
2
3
Time after index ICH (Years)
Edinburgh CT-only criteria
High
Intermediate
Low
Event type
Recurrent ICH

F


0.5
0.4
0.3
0.2
0.1
0.0
Probability
0
1
2
3
Time after index ICH (Years)
Event type
Death
Edinburgh CT-only criteria
High
Intermediate
Low

G



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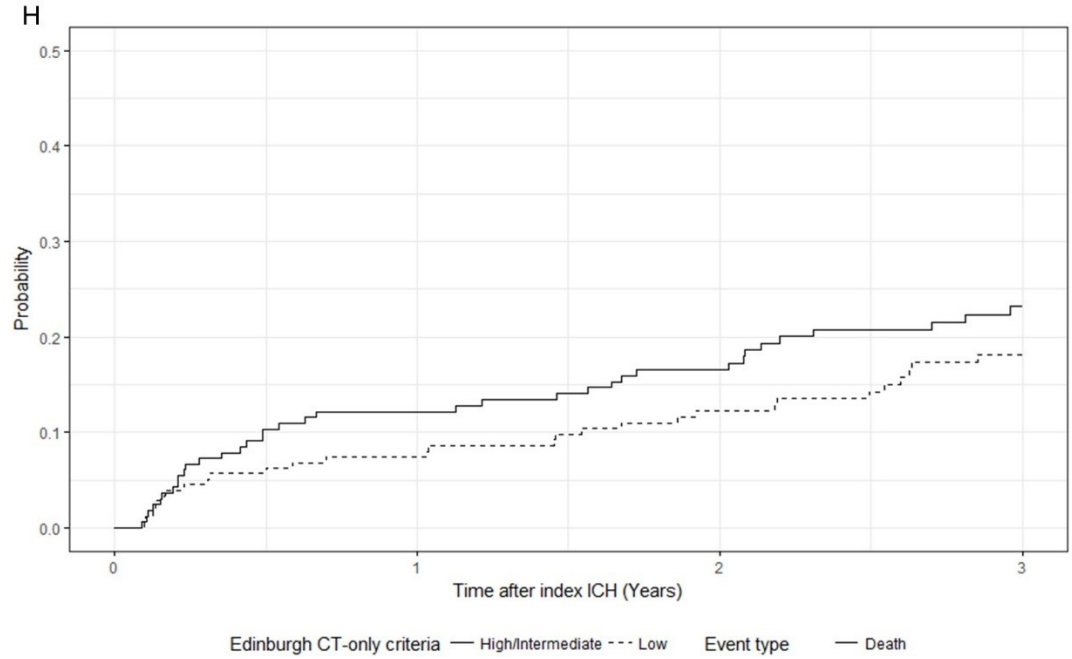


CAA = cerebral amyloid angiopathy. CROMIS = clinical relevance of microbleeds in stroke. CT = computed tomography. ICH = intracerebral haemorrhage. SVD = small vessel disease.

Table 10.8 Univariable sub-distribution and cause-specific hazard models for recurrent ICH and death in 342 CROMIS-2 participants with first-ever SVD-associated lobar ICH

Variable	Sub-distribution hazard model						Cause-specific hazard model					
	Recurrent ICH			Death			Recurrent ICH			Death		
	Hazard ratio		p value	Hazard ratio		p value	Hazard ratio		p value	Hazard ratio		p value
Sex												
Female	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Male	0.64	(0.29-1.40)	0.265	0.70	(0.43-1.14)	0.153	0.62	(0.28-1.38)	0.243	0.68	(0.42-1.11)	0.121
CT SVD score												
0	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
1, 2, 3	2.41	(1.07-5.44)	0.033	1.92	(1.17-3.14)	0.010	2.52	(1.12-5.71)	0.026	2.02	(1.24-3.31)	0.005
Edinburgh CT-only CAA criteria												
Low	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Intermediate	1.07	(0.43-2.66)	0.876	1.44	(0.85-2.43)	0.175	1.15	(0.46-2.85)	0.767	1.46	(0.86-2.46)	0.158
High	2.17	(0.80-5.89)	0.127	1.17	(0.56-2.43)	0.670	2.15	(0.79-5.81)	0.132	1.24	(0.59-2.62)	0.574
Edinburgh CT-only CAA criteria												
Low	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Intermediate/high	1.37	(0.62-3.01)	0.431	1.36	(0.84-2.21)	0.215	1.43	(0.65-3.16)	0.371	1.40	(0.86-2.28)	0.181

Data are hazard ratio (95%CI). CAA – cerebral amyloid angiopathy. CROMIS = clinical relevance of microbleeds in stroke. CT = computed tomography. ICH = intracerebral haemorrhage. SVD = small vessel disease.

Table 10.9 Multivariable sub-distribution and cause-specific hazard models for recurrent ICH and death in 342 CROMIS-2 participants with first-ever SVD-associated lobar ICH

	Sub-distribution hazard model						Cause-specific hazard model					
	Recurrent ICH			Death			Recurrent ICH			Death		
	Hazard ratio		p value	Hazard ratio		p value	Hazard ratio		p value	Hazard ratio		p value
CT SVD score												
0	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
1, 2, 3	2.37	(1.05-5.35)	0.038	1.91	(1.16-3.13)	0.011	2.49	(1.10-5.64)	0.029	2.01	(1.23-3.30)	0.006
Edinburgh CT-only CAA criteria												
Low	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Intermediate	1.08	(0.43-2.68)	0.872	1.41	(0.84-2.38)	0.194	1.14	(0.46-2.83)	0.782	1.44	(0.85-2.44)	0.171
High	2.10	(0.77-5.71)	0.147	1.13	(0.54-2.35)	0.754	2.09	(0.77-5.65)	0.147	1.21	(0.57-2.55)	0.621

Data are hazard ratio (95%CI). CAA – cerebral amyloid angiopathy. CROMIS = clinical relevance of microbleeds in stroke. CT = computed tomography. ICH = intracerebral haemorrhage. SVD = small vessel disease.

LATCH and CROMIS-2 meta-analysis

Two-step random-effects meta-analysis of the LATCH and CROMIS-2 multivariable models showed that participants classified as high risk on the Edinburgh CT-only diagnostic criteria had a higher risk of recurrent ICH compared with the low-risk group (subdistribution hazard ratio 2.99, 95%CI 1.08-8.27, Figure 10.8). There was no significant association between intermediate risk on the Edinburgh CT-only criteria or CT SVD score 1, 2 or 3 and recurrent ICH risk (Subdistribution hazard ratio 1.74, 95%CI 0.86-3.51).

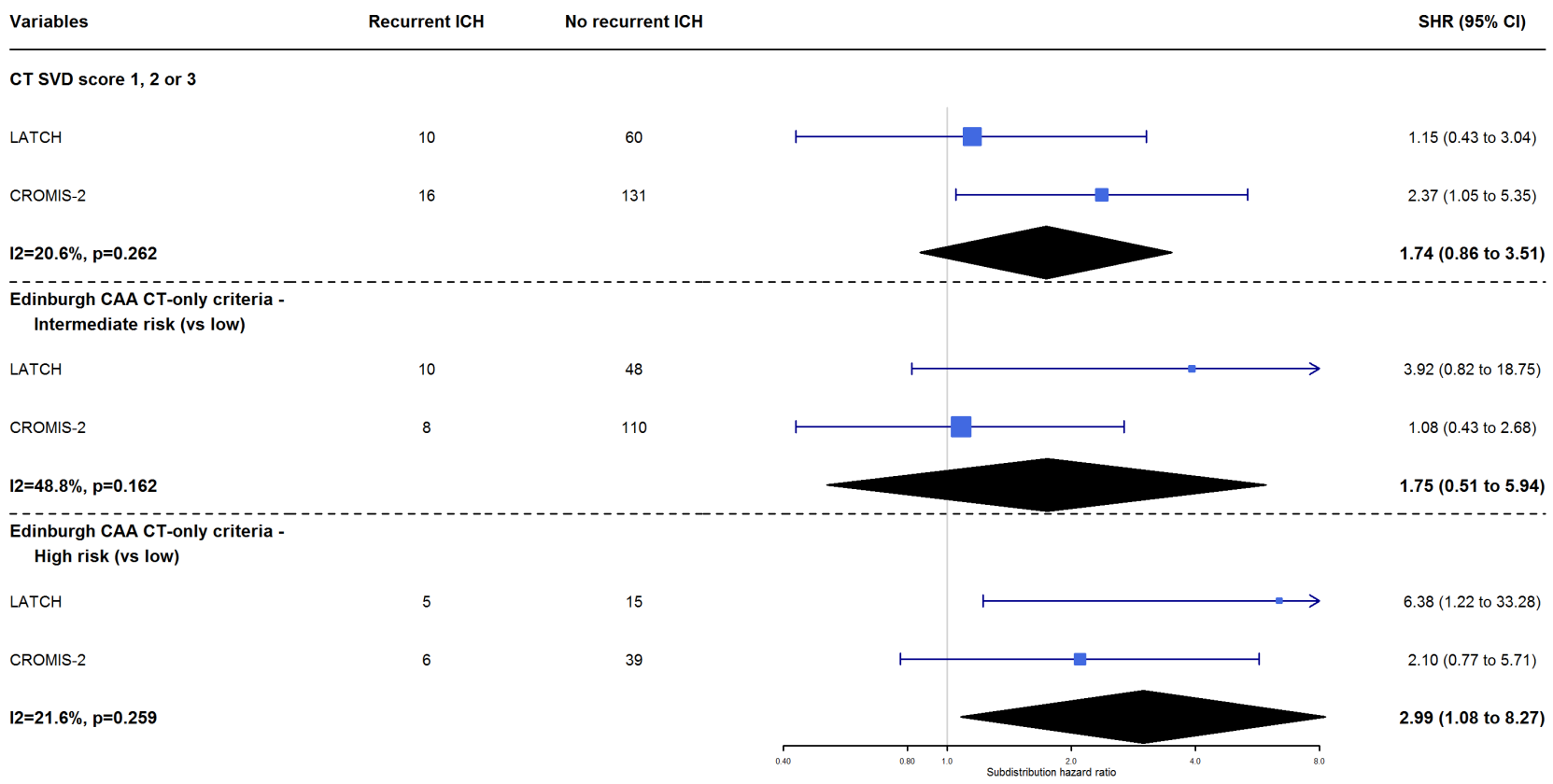
I performed a secondary analysis by pooling the LATCH and CROMIS-2 data (462 participants with first-ever SVD-associated lobar ICH) and then fitting multivariable models. This resulted in a similar direction and magnitude of associations with recurrent ICH as the two-step meta-analysis (Table 10.10). High risk on the Edinburgh CT-only diagnostic criteria was associated with increased risk (subdistribution hazard ratio 2.98, 95%CI 1.33-6.67) and rate (cause-specific hazard ratio 3.14, 95%CI 1.40-7.00) of recurrent ICH. CT SVD score of 1, 2 or 3 had a borderline significant association with recurrent ICH in the subdistribution hazard model, and a significant association in the cause-specific hazard model (HR 2.02, 95%CI 1.08-3.76).

Secondary analyses

I performed pre-specified secondary analyses assessing the effect of other relevant variables, including the individual components of the Edinburgh CT-only diagnostic criteria, on the risk of recurrent ICH or death during follow up in LATCH and CROMIS-2 participants with SVD-associated lobar ICH.

The 3-year cumulative incidence of recurrent ICH was higher in those with a previous symptomatic ICH, as well as those who were not taking an antihypertensive drug at hospital discharge although these differences were not statistically significant (Figure 10.9 and Table 10.11). Those with subarachnoid haemorrhage on the diagnostic non-contrast brain CT had a significantly higher cumulative incidence of recurrent ICH. The cumulative incidence of recurrent ICH was also higher in those with finger-like projections, although this difference did not reach significance.

Figure 10.8 Pooled risks of recurrent intracerebral haemorrhage during follow up in LATCH and CROMIS-2 participants with first-ever lobar ICH



Weights from random effects analysis are shown by the point estimate area. CAA = cerebral amyloid angiopathy. CI = confidence interval. CROMIS = clinical relevance of microbleeds in stroke. CT – computed tomography. LATCH = Lothian audit of the treatment of cerebral haemorrhage. SHR = subdistribution hazard ratio. SVD = small vessel disease.

Table 10.10 Secondary analysis: Multivariable sub-distribution and cause-specific hazard models for recurrent ICH and death in 462 LATCH and CROMIS-2 participants with first-ever SVD-associated lobar ICH

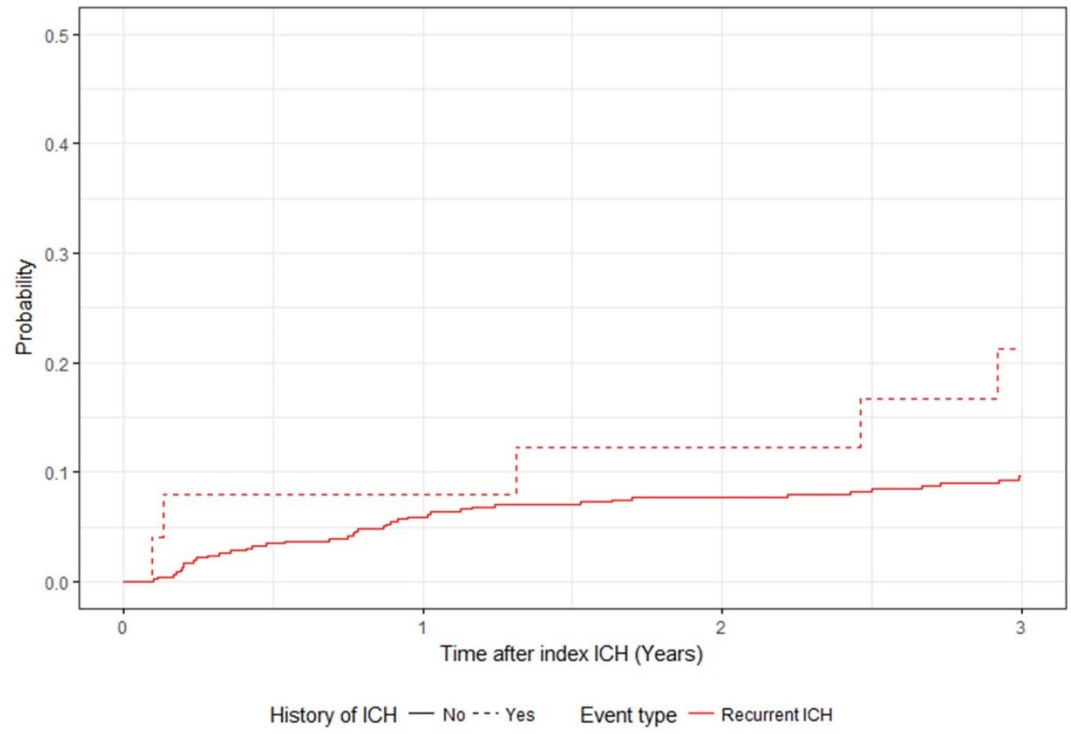
	Sub-distribution hazard model						Cause-specific hazard model					
	Recurrent ICH			Death			Recurrent ICH			Death		
	Hazard ratio		p value	Hazard ratio		p value	Hazard ratio		p value	Hazard ratio		p value
CT SVD score												
0	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
1, 2, 3	1.86	(1.00-3.45)	0.050	1.88	(1.28-2.76)	0.001	2.02	(1.08-3.76)	0.027	2.00	(1.36-2.93)	<0.001
Edinburgh CT-only CAA criteria												
Low	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Intermediate	1.75	(0.86-3.56)	0.124	1.26	(0.85-1.89)	0.252	1.82	(0.89-3.72)	0.100	1.32	(0.88-1.98)	0.178
High	2.98	(1.33-6.67)	0.008	1.17	(0.67-2.03)	0.590	3.14	(1.40-7.00)	0.005	1.35	(0.78-2.36)	0.287

Data are hazard ratio (95%CI). CAA = cerebral amyloid angiopathy. CROMIS = clinical relevance of microbleeds in stroke. CT = computed tomography. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. SVD = small vessel disease.

Figure 10.9 Cumulative incidence of recurrent ICH in SVD-associated lobar ICH in the pooled LATCH and CROMIS-2 data

A. History of previous ICH. B. History of hypertension. C. History of dementia. D-F. Antiplatelet, anticoagulant and antihypertensive drug use on discharge respectively. G. Subarachnoid haemorrhage. H. Finger-like projections.

A



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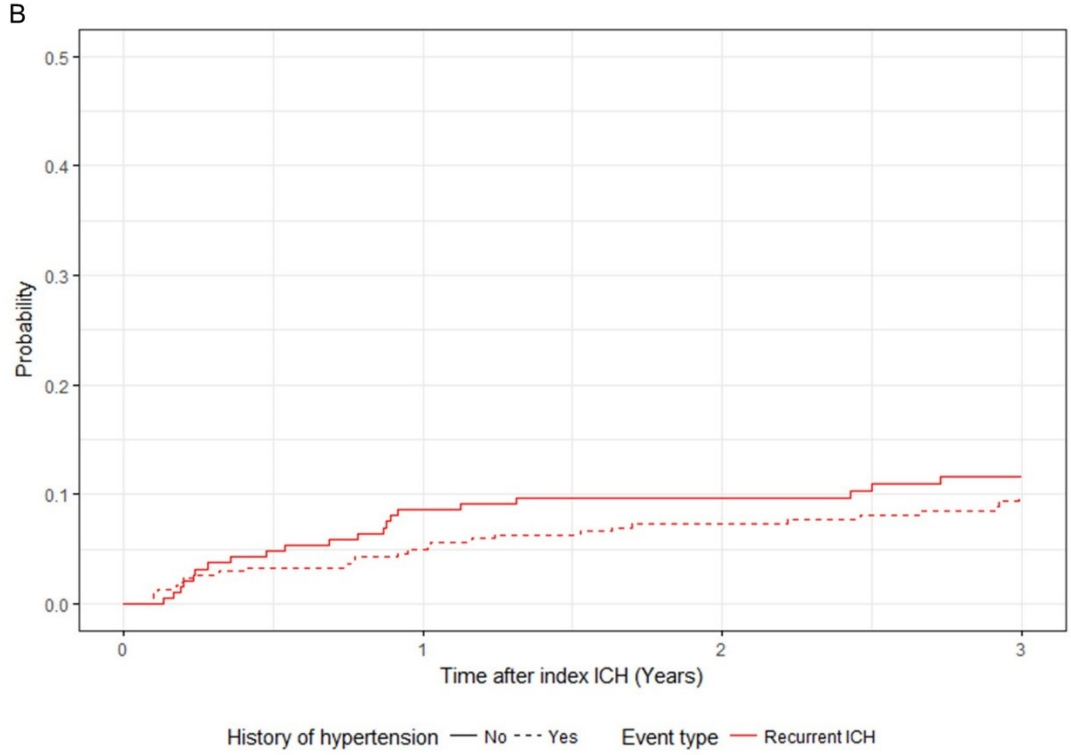

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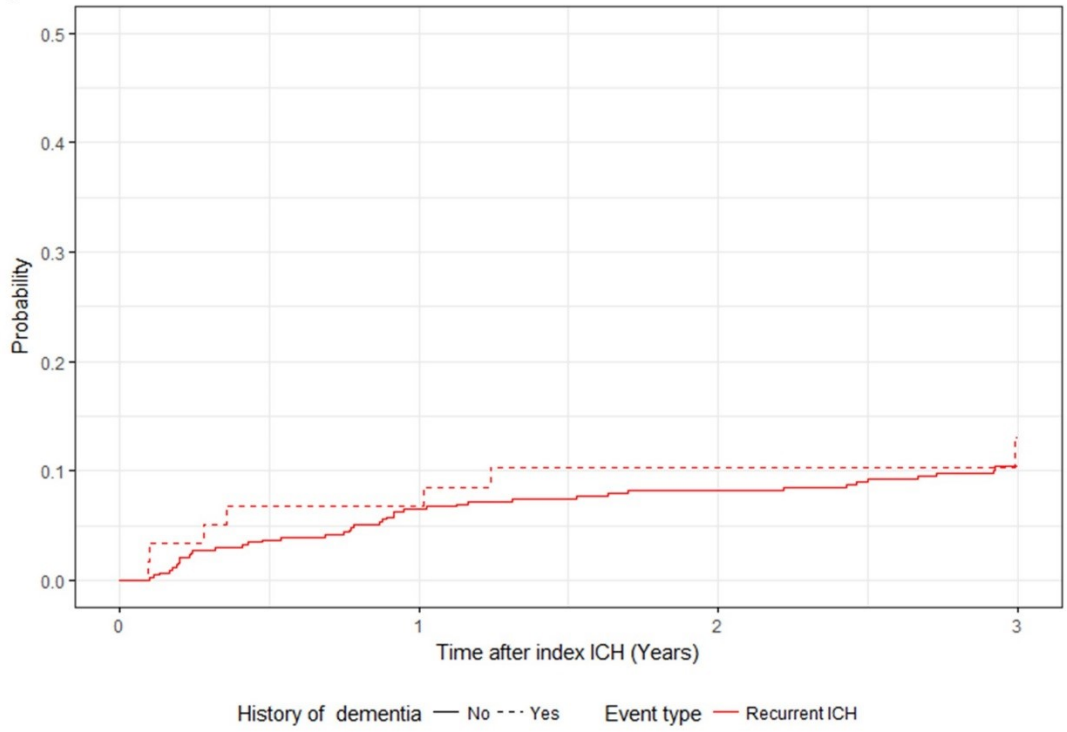
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Probability
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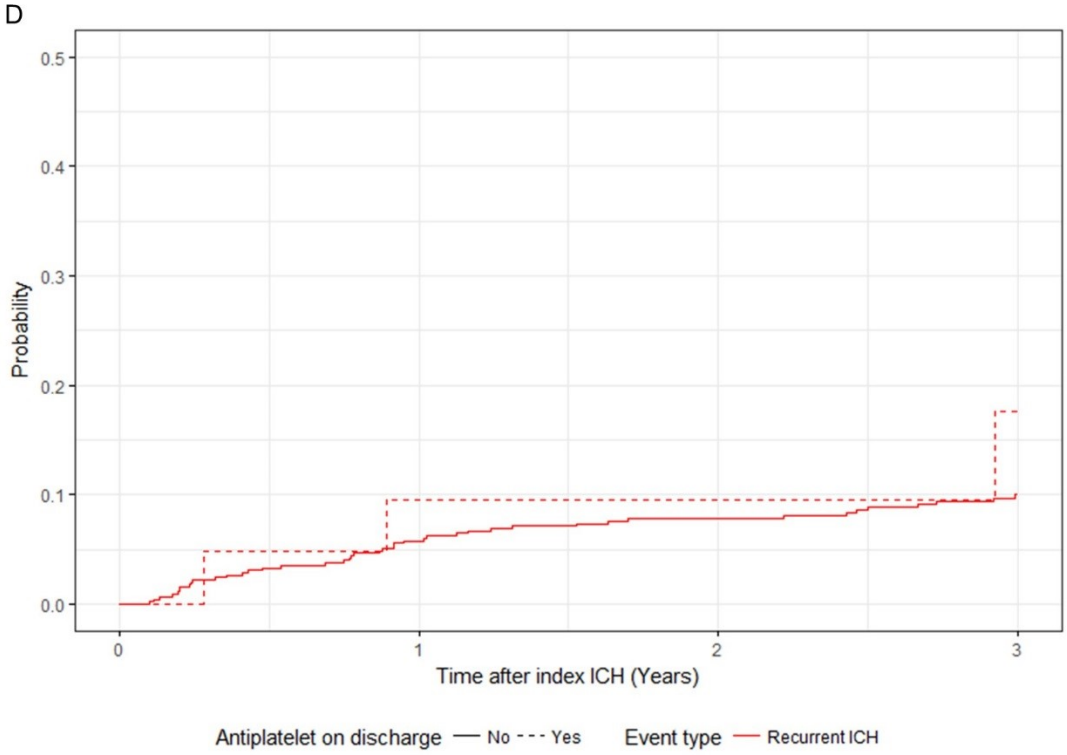
2
3
Time after index ICH (Years)
History of dementia No Yes
Event type Recurrent ICH

D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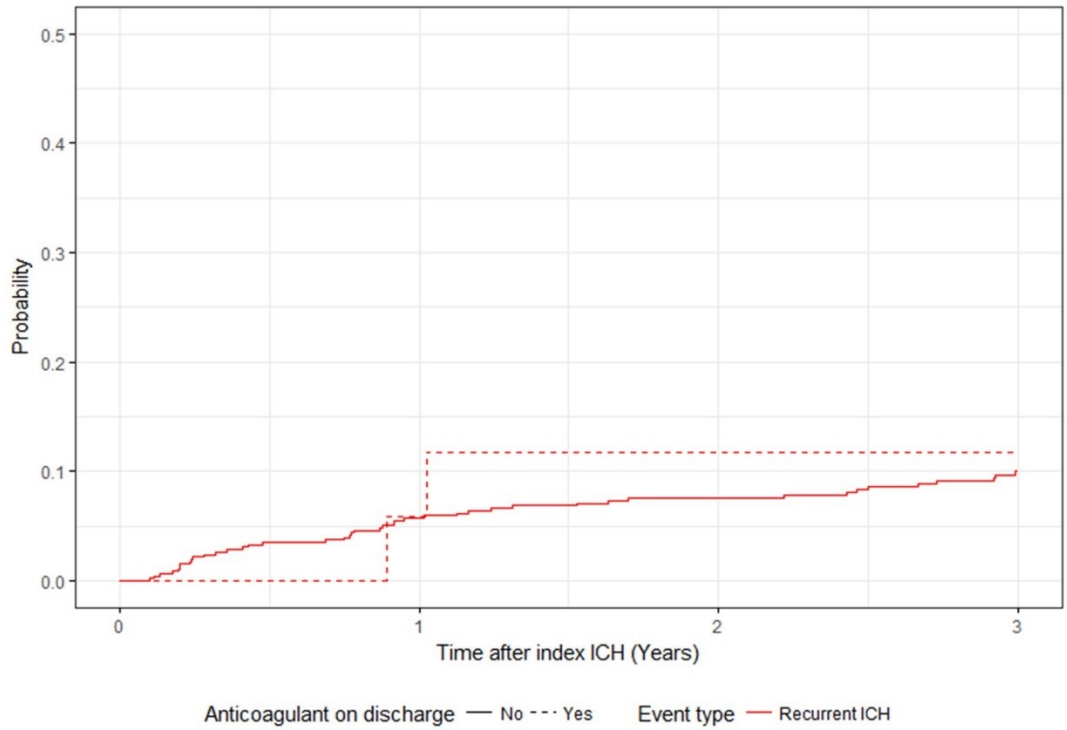
0.2
0.1
0.0
Probability
0
1
2
3
Time after index ICH (Years)
Antiplatelet on discharge No Yes
Event type Recurrent ICH

E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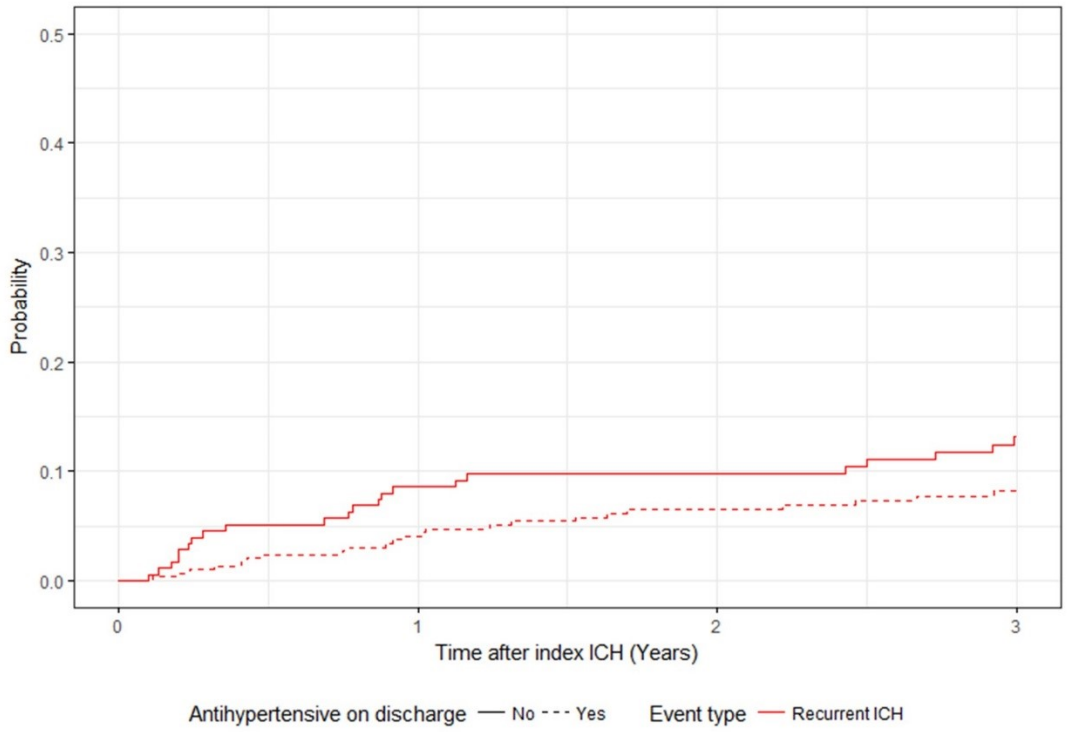
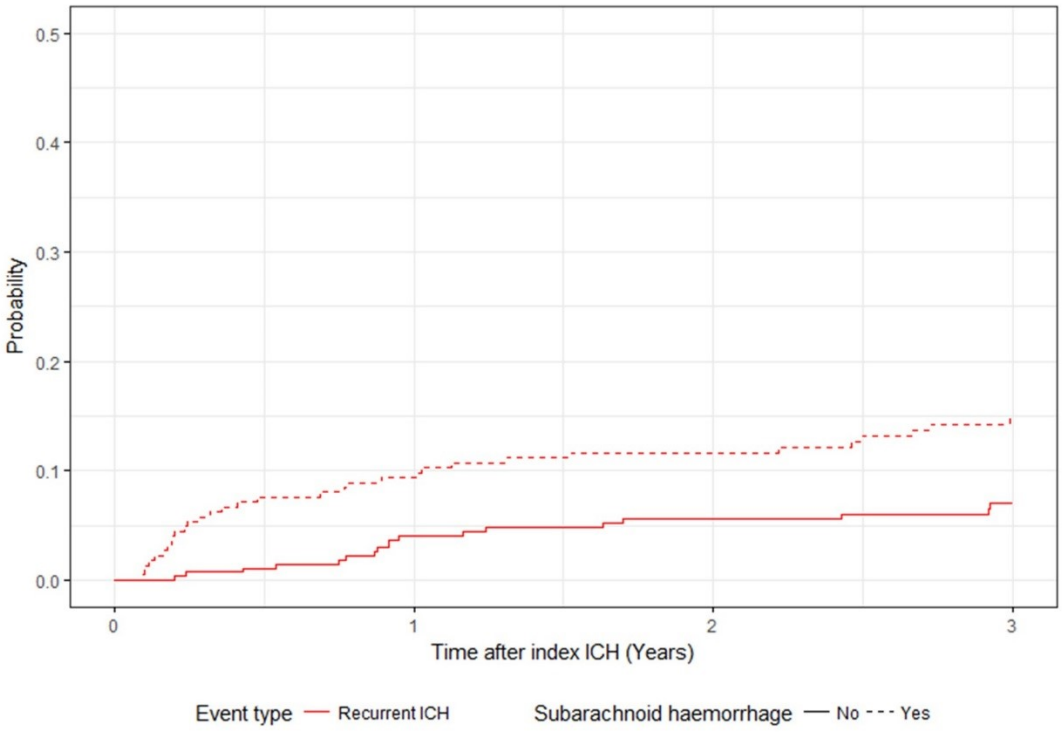
0.3
0.2
0.1
0.0
Probability
0
1
2
3
Time after index ICH (Years)
Anticoagulant on discharge
No
Yes
Event type
Recurrent ICH

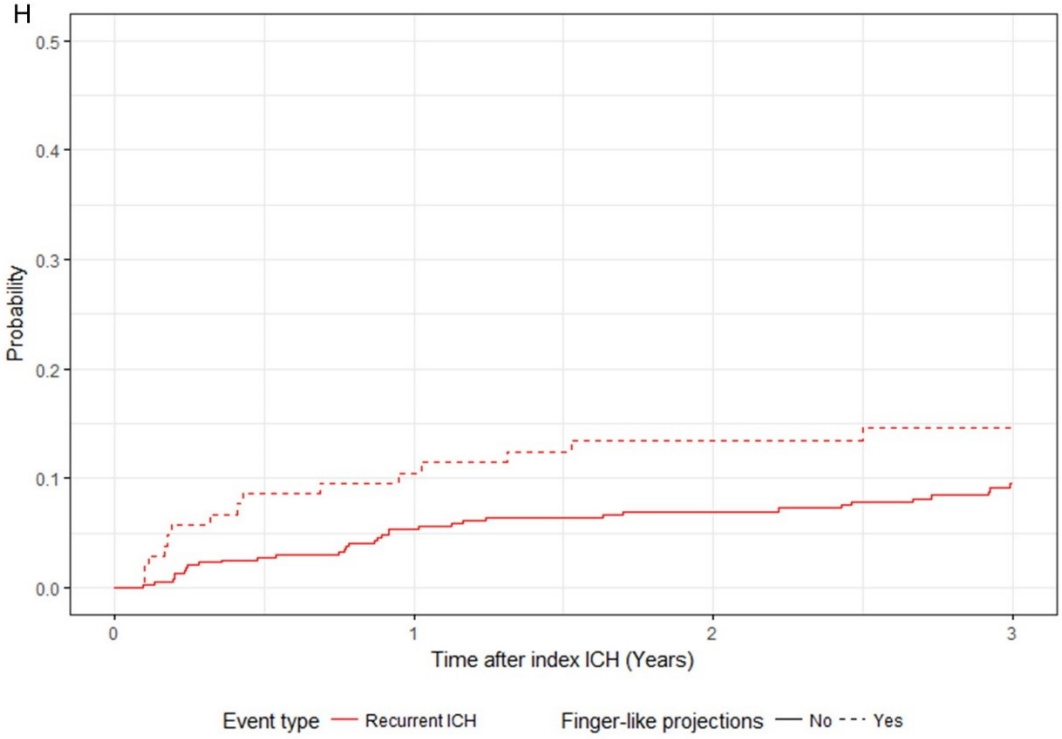
F


0.5
0.4
0.3
0.2
0.1
0.0
Probability
0
1
2
3
Time after index ICH (Years)
Antihypertensive on discharge
No
Yes
Event type
Recurrent ICH

G



H



CROMIS = clinical relevance of microbleeds in stroke. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. SVD = small vessel disease.

Table 10.11 Secondary analysis: Three year cumulative incidence of recurrent ICH and death in pooled LATCH and CROMIS-2 participants with SVD-associated lobar ICH

	Number of participants	Recurrent ICH			Death		
		Number of events	3 year cumulative incidence rate	p value	Number of events	3 year cumulative incidence rate	p value
History of ICH							
No	462	42	9.6% (7.1-12.6)	0.078	111	25.6% (21.5-29.8)	0.900
Yes	25	5	21.2% (7.4-39.7)		6	24.9% (9.8-43.6)	
History of hypertension							
No	189	21	11.6% (7.4-16.8)	0.462	38	21.4% (15.6-27.7)	0.049
Yes	306	28	9.8% (6.7-13.6)		85	29.4% (24.2-34.8)	
History of dementia							
No	437	43	10.4% (7.7-13.6)	0.575	98	23.8% (19.7-27.9)	<0.001
Yes	59	7	13.0% (5.5-23.9)		26	48.1% (33.6-61.2)	
Antiplatelet on discharge							
No	457	43	10.0% (7.4-13.1)	0.455	109	25.4% (21.3-29.6)	0.722
Yes	21	3	17.6% (3.5-40.4)		6	34.2% (12.7-57.2)	
Anticoagulant on discharge							
No	460	43	10.0% (7.4-13.1)	0.733	114	26.4% (22.3-30.7)	0.084
Yes	17	2	11.8% (1.8-32.0)		1	5.9% (0.3-24.2)	
Antihypertensive on discharge							
No	177	22	13.1% (8.5-18.8)	0.088	45	26.4% (20.0-33.2)	0.464
Yes	300	23	8.2% (5.3-11.8)		70	25.5% (20.4-30.8)	
Subarachnoid haemorrhage							
No	277	18	7.0% (4.3-10.6)	0.005	63	24.5% (19.3-29.9)	0.322
Yes	225	32	14.8% (10.4-19.8)		61	28.3% (22.4-34.5)	
Finger-like projections							
No	397	35	9.4% (6.7-12.7)	0.085	96	25.7% (21.4-30.3)	0.591
Yes	105	15	14.6% (8.5-22.1)		28	28.1% (19.5-37.3)	

Data are % (95%CI). CROMIS = clinical relevance of microbleeds in stroke. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. SVD = small vessel disease.

Multivariable models adjusting for age and sex, history of previous ICH, antihypertensive drug use at hospital discharge, CT SVD score and the Edinburgh CT-only diagnostic criteria are shown in Table 10.12. I excluded 42 participants due to missing data, leaving 460 participants, of whom 42 had a recurrent ICH, and 108 died during follow up. High risk on the Edinburgh CT-only criteria was associated with an increased risk (subdistribution hazard ratio 2.81, 95%CI 1.17-6.72) and rate (cause-specific hazard ratio 2.79, 95%CI 1.19-6.57) of recurrent ICH relative to the low-risk category. Intermediate risk on the Edinburgh CT-only criteria was associated with a borderline significant increased risk of recurrent ICH (subdistribution hazard ratio 2.04, 95%CI 1.00-4.16) and a significantly increased rate of recurrent ICH (cause-specific hazard ratio 2.08, 95%CI 1.03-4.22).

Finally, I refitted the above models using subarachnoid haemorrhage and finger-like projections in place of the Edinburgh CT-only criteria. In these models (Table 10.13), only subarachnoid haemorrhage was associated with an increased risk (subdistribution hazard ratio 2.15, 95%CI 1.13-4.12) and rate (cause-specific hazard ratio 2.18, 95%CI 1.13-4.21) of recurrent ICH.

Confounders

The proportion of first-ever SVD-associated lobar ICH LATCH participants on an antiplatelet, anticoagulant or antihypertensive drug at hospital discharge was similar between those with and without a recurrent ICH (Table 10.14), as well as the Edinburgh CT-only risk categories (Table 10.15).

In CROMIS-2, the proportion of participants with a first-ever SVD-associated lobar ICH on an antihypertensive drug at hospital discharge was lower in those who had a recurrent ICH (54%) compared to those without a recurrent ICH (72%) (Table 10.16), and lower in those classified as high risk on the Edinburgh CT-only criteria (61%) compared to low risk (75%) (Table 10.17). The proportion taking an antiplatelet or anticoagulant drug at hospital discharge was similar across the groups.

Table 10.12 Secondary analysis: Multivariable subdistribution and cause-specific hazard models for recurrent ICH and death in 460 LATCH and CROMIS-2 participants with SVD-associated lobar ICH (42 recurrent ICH, 108 deaths)

	Subdistribution hazard model						Cause-specific hazard model					
	Recurrent ICH			Death			Recurrent ICH			Death		
	Hazard ratio		p value	Hazard ratio		p value	Hazard ratio		p value	Hazard ratio		p value
Age (per 10 year increase)	1.08	(0.79-1.48)	0.619	2.34	(1.83-2.99)	<0.001	1.21	(0.86-1.71)	0.274	2.36	(1.84-3.01)	<0.001
Sex												
Female	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Male	0.74	(0.40-1.35)	0.321	1.26	(0.85-1.85)	0.254	0.44	(0.41-1.45)	0.421	1.19	(0.81-1.76)	0.372
History of ICH												
No	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Yes	2.32	(0.83-6.49)	0.109	1.36	(0.52-3.52)	0.531	2.54	(0.89-7.28)	0.082	1.38	(0.55-3.46)	0.488
Antihypertensive on discharge												
No	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Yes	0.67	(0.37-1.24)	0.204	0.75	(0.50-1.11)	0.148	0.60	(0.32-1.10)	0.096	0.74	(0.50-1.09)	0.132
CT SVD score												
0	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
1, 2, 3	1.59	(0.83-3.05)	0.165	1.23	(0.82-1.85)	0.317	1.64	(0.86-3.14)	0.136	1.27	(0.84-1.91)	0.253
Edinburgh CT-only CAA criteria												
Low	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Intermediate	2.04	(1.00-4.16)	0.051	1.25	(0.84-1.67)	0.279	2.08	(1.03-4.22)	0.042	1.32	(0.88-1.98)	0.181
High	2.81	(1.17-6.72)	0.021	0.91	(0.50-1.67)	0.759	2.79	(1.19-6.57)	0.019	1.03	(0.56-1.91)	0.927

Data are hazard ratio (95%CI). CAA – cerebral amyloid angiopathy. CROMIS = clinical relevance of microbleeds in stroke. CT = computed tomography. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. SVD = small vessel disease.

Table 10.13 Secondary analysis: Multivariable subdistribution and cause-specific hazard models for recurrent ICH and death in 460 LATCH and CROMIS-2 participants with SVD-associated lobar ICH (42 recurrent ICH, 108 deaths)

	Subdistribution hazard model						Cause-specific hazard model					
	Recurrent ICH			Death			Recurrent ICH			Death		
	Hazard ratio		p value	Hazard ratio		p value	Hazard ratio		p value	Hazard ratio		p value
Age (per 10 year increase)	1.09	(0.80-1.49)	0.583	2.37	(1.85-3.04)	<0.001	1.22	(0.87-1.73)	0.252	2.39	(1.86-3.06)	<0.001
Sex												
Female	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Male	0.75	(0.41-1.37)	0.345	1.28	(0.87-1.90)	0.216	0.79	(0.42-1.49)	0.475	1.22	(0.83-1.80)	0.317
History of ICH												
No	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Yes	2.27	(0.82-6.30)	0.116	1.32	(0.51-3.43)	0.564	2.48	(0.87-7.08)	0.090	1.35	(0.54-3.37)	0.522
Antihypertensive on discharge												
No	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Yes	0.69	(0.38-1.26)	0.227	0.75	(0.51-1.10)	0.144	0.60	(0.33-1.11)	0.106	0.74	(0.50-1.10)	0.134
CT SVD score												
0	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
1, 2, 3	1.55	(0.80-2.98)	0.192	1.18	(0.78-1.79)	0.422	1.59	(0.83-3.07)	0.163	1.23	(0.81-1.85)	0.336
Subarachnoid haemorrhage												
No	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Yes	2.15	(1.13-4.12)	0.021	1.20	(0.82-1.77)	0.355	2.18	(1.13-4.21)	0.020	1.27	(0.86-1.88)	0.223
Finger-like projections												
No	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Yes	1.30	(0.64-2.65)	0.468	0.82	(0.49-1.38)	0.450	1.27	(0.62-2.62)	0.509	0.87	(0.53-1.43)	0.580

Data are hazard ratio (95%CI). CAA – cerebral amyloid angiopathy. CROMIS = clinical relevance of microbleeds in stroke. CT = computed tomography. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage. SVD = small vessel disease.

Table 10.14 Medication use at hospital discharge in LATCH participants with first-ever lobar ICH stratified by the outcome of recurrent ICH during follow-up

	No recurrent ICH (n=103)	Recurrent ICH (n=17)
Antiplatelet at discharge*	2 (2%)	1 (7%)
Anticoagulant at discharge*	2 (2%)	2 (14%)
Antihypertensive at discharge*	44 (44%)	7 (50%)

* Data is not available for six LATCH participants with first-ever lobar ICH who survived more than 30 days but died before hospital discharge. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage.

Table 10.15 Medication use at hospital discharge in LATCH participants with first-ever lobar ICH stratified by Edinburgh CT-only criteria classification

	Low risk CT-only Edinburgh CAA criteria (n=42)	Intermediate risk CT-only Edinburgh CAA criteria (n=58)	High risk CT-only Edinburgh CAA criteria (n=20)
Antiplatelet at discharge*	1 (2%)	1 (2%)	1 (7%)
Anticoagulant at discharge*	0 (0%)	2 (4%)	2 (13%)
Antihypertensive at discharge*	21 (50%)	22 (39%)	8 (53%)

* Data is not available for six LATCH participants with first-ever lobar ICH who survived more than 30 days but died before hospital discharge. CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. LATCH = Lothian audit of the treatment of cerebral haemorrhage.

Table 10.16 Medication use at hospital discharge in CROMIS-2 participants with first-ever lobar ICH stratified by the outcome of recurrent ICH during follow-up

	No recurrent ICH (n=317)	Recurrent ICH (n=25)
Antiplatelet at discharge*	14 (5%)	2 (8%)
Anticoagulant at discharge†	12 (4%)	0 (0%)
Antihypertensive at discharge†	217 (72%)	13 (54%)

* Data is not available for 15 CROMIS-2 participants. † Data is not available for 16 CROMIS-2 participants. CROMIS = clinical relevance of microbleeds in stroke. ICH = intracerebral haemorrhage.

Table 10.17 Medication use at hospital discharge in CROMIS-2 participants with first-ever lobar ICH stratified by Edinburgh CT-only criteria classification

	Low risk CT-only Edinburgh CAA criteria (n=177)	Intermediate risk CT-only Edinburgh CAA criteria (n=120)	High risk CT-only Edinburgh CAA criteria (n=45)
Antiplatelet at discharge*	7 (4%)	7 (6%)	2 (5%)
Anticoagulant at discharge†	6 (4%)	5 (4%)	1 (2%)
Antihypertensive at discharge†	126 (75%)	79 (68%)	25 (61%)

* Data is not available for 15 CROMIS-2 participants. † Data is not available for 16 CROMIS-2 participants. CAA = cerebral amyloid angiopathy. CROMIS = clinical relevance of microbleeds in stroke. CT = computed tomography. ICH = intracerebral haemorrhage.

10.4.2 Edinburgh CT-APOE diagnostic criteria for CAA-associated lobar ICH study

10.4.2.1 Flow of patients

LINCHPIN

Three hundred and fifty participants with ICH presumed secondary to SVDs consented to the LINCHPIN study between 1st June 2010 and 31st May 2016 inclusive. Two hundred and eighty-two had both a diagnostic non-contrast brain CT and APOE genotyping, of whom 197 survived at least 30 days without having a recurrent ICH (Figure 10.10).

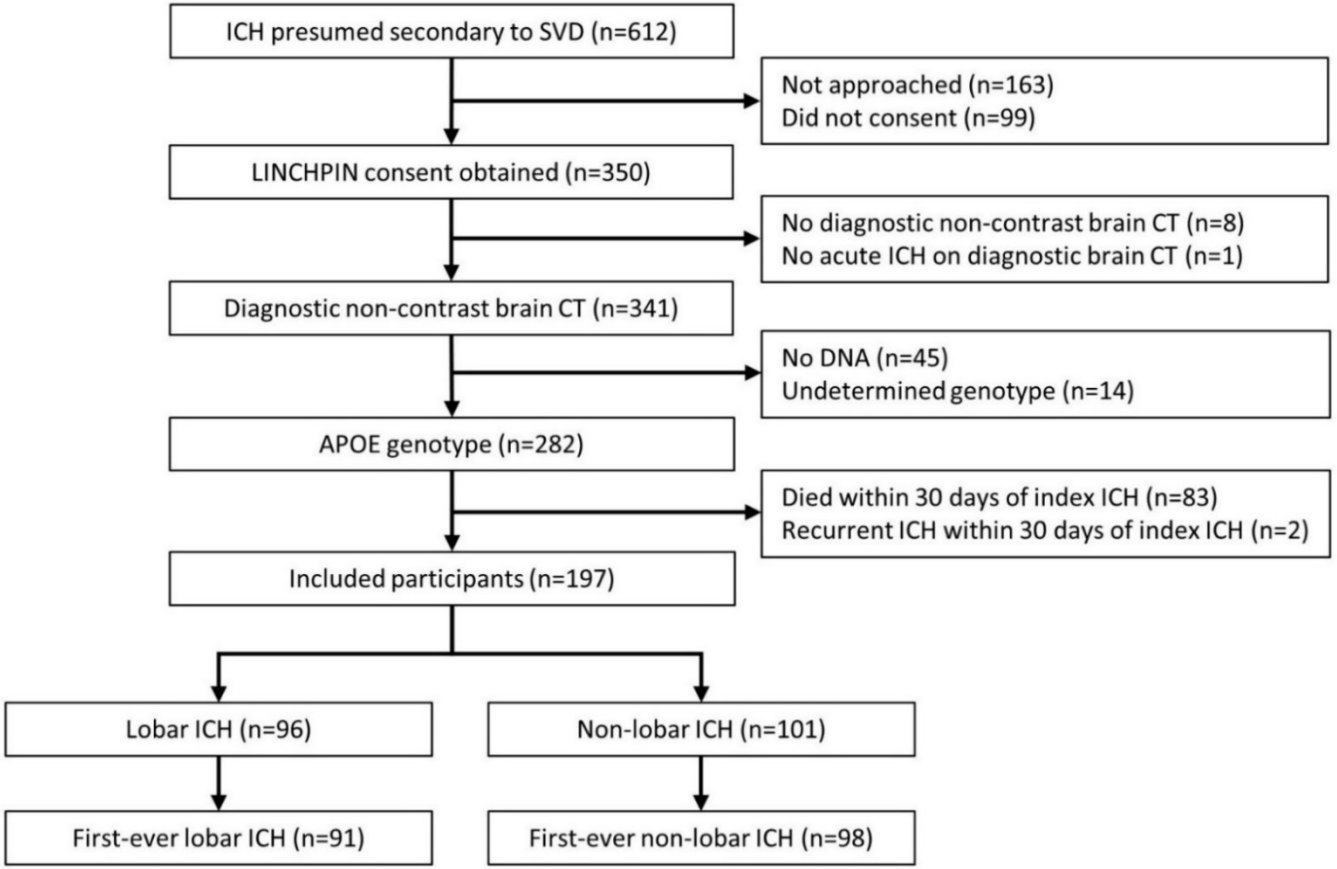
CROMIS-2-DNA

Of the 1026 participants with an ICH presumed secondary to SVDs in the CROMIS-2 study, 861 had both a diagnostic non-contrast brain CT and APOE genotyping. Four had missing data for the outcome, while 51 died or had a recurrent ICH within 30 days of their index event. Therefore, I included 806 participants, of whom 314 had a lobar ICH (Figure 10.11).

10.4.2.2 Comparison of LINCHPIN and CROMIS-2-DNA participants with first-ever lobar ICH

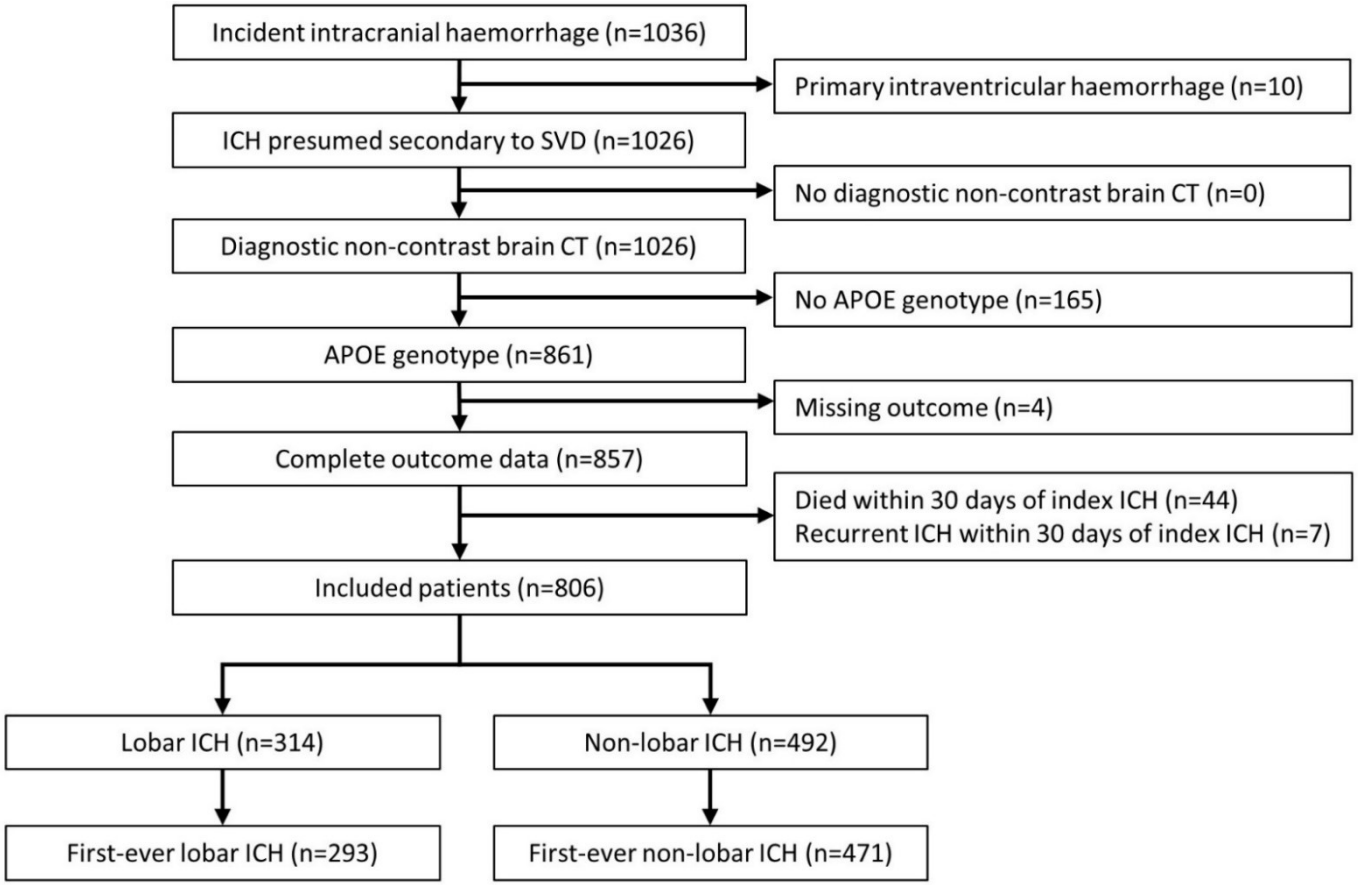
Participants in LINCHPIN and CROMIS-2-DNA were similar in age and had a similar frequency of finger-like projections (Table 10.18 and Table 10.19). CROMIS-2-DNA had a significantly higher proportion of male participants. The frequency of pre-ICH diabetes, atrial fibrillation and hyperlipidaemia were higher in CROMIS-2-DNA. There was more frequent pre-ICH use of anticoagulant and antihypertensive drugs in CROMIS-2-DNA but less frequent pre-ICH antiplatelet drug use. Subarachnoid haemorrhage and CT measures of SVDs were more frequent in LINCHPIN, while more CROMIS-2-DNA participants were classified as low risk by the Edinburgh CT-APOE diagnostic criteria. A higher proportion of LINCHPIN participants had a recurrent ICH or died during follow up.

Figure 10.10 Flowchart of SVD-associated ICH participants in the LINCHPIN study who had a diagnostic non-contrast brain CT, APOE genotyping and survived at least 30 days.



APOE = apolipoprotein E. CT = computed tomography. DNA= deoxyribonucleic acid. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. SVD = small vessel disease.

Figure 10.11 Flowchart of SVD-associated ICH participants in the CROMIS-2 study who had a diagnostic non-contrast brain CT, APOE genotyping and survived at least 30 days.



APOE = apolipoprotein E. CROMIS = clinical relevance of microbleeds in stroke. CT = computed tomography. DNA= deoxyribonucleic acid. ICH = intracerebral haemorrhage. SVD = small vessel disease.

Table 10.18 Baseline clinical features in first-ever SVD-associated lobar ICH participants in the LINCHPIN and CROMIS-2-DNA cohorts

	LINCHPIN first-ever lobar ICH (n=91)	CROMIS-2-DNA first-ever lobar ICH (n=293)	p value
Age (years); median (IQR)	78 (71-83)	76 (69-83)	0.500
Unknown	*0*	*1*	
Sex			
Female	58 (64)	134 (46)	0.003
Male	33 (36)	159 (54)	
Co-morbidities			
Hypertension	51 (56)	181 (63)	0.231
Unknown	*0*	*6*	
Ischaemic stroke	5 (6)	35 (12)	0.078
Unknown	*0*	*0*	
Transient ischaemic attack	7 (8)	39 (14)	0.124
Unknown	*0*	*10*	
Dementia	6 (7)	29 (10)	0.344
Unknown	*0*	*5*	
Diabetes	9 (10)	55 (19)	0.043
Unknown	*0*	*3*	
Atrial fibrillation	18 (20)	97 (36)	0.004
Unknown	*0*	*25*	
Myocardial infarction	8 (9)	24 (8)	0.887
Unknown	*0*	*3*	
Hyperlipidaemia	10 (11)	139 (49)	<0.001
Unknown	*0*	*10*	
Smoking status			
Current	19 (22)	22 (8)	
Ex-smoker	27 (31)	109 (39)	0.001
Never	41 (47)	150 (53)	
Unknown	*4*	*12*	
Medications on admission			
Antiplatelet drug(s)	36 (40)	75 (26)	0.010
Unknown	*0*	*0*	
Anticoagulant drug(s)	13 (14)	122 (42)	<0.001
Unknown	*0*	*1*	
Antihypertensive drug(s)	39 (43)	174 (60)	0.005
Unknown	*0*	*1*	
Admission GCS; median (IQR)	14 (13-15)	15 (14-15)	0.001
Unknown	*1*	*5*	
Medications on discharge			
Antiplatelet drug(s)*	2 (2)	12 (4)	
Died during admission	*4*	*?*	0.533
Unknown	*0*	*12*	
Anticoagulant drug(s)*	3 (3)	12 (4)	
Died during admission	*4*	*?*	1.000
Unknown	*0*	*13*	
Antihypertensive drug(s)	48 (55)	199 (71)	
Died during admission	*4*	*?*	0.006
Unknown	*0*	*13*	
Outcome			
Recurrent ICH	14 (15)	24 (8)	
Death	27 (30)	51 (17)	0.002
Censored	50 (55)	218 (74)	

Data are n (%) or median (IQR). * = Fisher's exact test. CROMIS = clinical relevance of microbleeds in stroke. DNA = deoxyribonucleic acid. GCS = Glasgow coma scale. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. SVD = small vessel disease.

Table 10.19 Non-contrast brain CT features and APOE genotype in first-ever SVD-associated lobar ICH participants in the LINCHPIN and CROMIS-2-DNA cohorts

	LINCHPIN first-ever lobar ICH (n=91)	CROMIS-2-DNA first-ever lobar ICH (n=293)	p value
APOE ε4 allele possession	39 (43)	90 (31)	0.032
APOE ε2 allele possession	27 (30)	72 (25)	0.332
Multiple acute ICH*	10 (11)	5 (2)	<0.001
Location of largest ICH*			
Frontal	37 (41)	45 (15)	<0.001
Parietal	25 (28)	134 (46)	
Temporal	14 (15)	24 (8)	
Occipital	15 (17)	87 (30)	
Insular	0 (0)	3 (1)	
ICH volume (ml); median (IQR)	19 (10-36)	14 (5-29)	0.007
Intraventricular haemorrhage	19 (21)	37 (13)	0.051
Subarachnoid haemorrhage	58 (64)	112 (38)	<0.001
Subdural haemorrhage*	9 (10)	5 (2)	0.001
Finger-like projections	18 (20)	66 (23)	0.570
Number of lacunes; median (IQR)	0 (0-0)	0 (0-0)	<0.001
Anterior white matter lucencies			
0	21 (23)	194 (66)	<0.001
1	56 (62)	57 (20)	
2	14 (15)	42 (14)	
Posterior white matter lucencies			
0	29 (32)	182 (62)	<0.001
1	23 (25)	37 (13)	
2	39 (43)	74 (25)	
Central atrophy			
0	36 (40)	41 (14)	<0.001
1	49 (54)	208 (71)	
2	6 (7)	44 (15)	
Cortical atrophy			
0	24 (26)	85 (29)	0.795
1	48 (53)	155 (53)	
2	19 (21)	53 (18)	
CT SVD score*			
0	38 (42)	169 (58)	0.012
1	32 (35)	87 (30)	
2	19 (21)	36 (12)	
3	2 (2)	1 (0)	
CT SVD score 1, 2 or 3	53 (58)	124 (42)	0.008
Edinburgh CT-APOE CAA criteria			
Low	22 (24)	110 (38)	0.004
Intermediate	33 (36)	116 (40)	
High	36 (40)	67 (23)	
Edinburgh CT-APOE CAA criteria Intermediate/high	69 (76)	183 (63)	0.019

Data are n (%) or median (IQR). * = Fisher's exact test. APOE = apolipoprotein E. CAA = cerebral amyloid angiopathy. CROMIS = clinical relevance of microbleeds in stroke. CT = computed tomography. DNA = deoxyribonucleic acid. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. SVD = small vessel disease.

10.4.2.3 Completeness of follow up

The median duration of follow-up in LINCHPIN was 1027 days (IQR 545-1095), with 97.7% completeness of follow-up. The median duration of follow-up in CROMIS-2-DNA was 1094 days (IQR 702-1094), with 95.8% completeness of follow-up.

10.4.2.4 Risk of recurrent ICH in participants with first-ever lobar ICH

LINCHPIN cohort

Fourteen of the 91 LINCHPIN participants with first-ever SVD-associated lobar ICH had a recurrent ICH, and 27 died during follow up.

The 3-year cumulative incidence rate for recurrent ICH and death according to sex, CT SVD score and Edinburgh CT-APOE diagnostic criteria are shown in Table 10.20 and Figure 10.12.

The 3 year cumulative incidence rate for recurrent ICH generally increased with the Edinburgh CT-APOE CAA criteria (low risk 4.5%, 95%CI 0.3-19.5); intermediate risk 13.1%, 95%CI 4.0-27.9; high risk 26.8%, 95%CI 12.7-43.2, p=0.063). The 3-year cumulative incidence of death was similar across the Edinburgh CT-only risk categories (Figure 10.12).

There was no significant change in the 3-year cumulative incidence of recurrent ICH with the CT SVD score. The cumulative incidence of death was higher in those with a CT SVD score of 1, 2 or 3 versus CT SVD score of 0, which was borderline significant (Table 10.20 and Figure 10.12).

The univariable subdistribution and cause-specific hazard models for sex, CT SVD score and Edinburgh CT-only diagnostic criteria are shown in Table 10.21. High risk on the Edinburgh CT-APOE criteria increased the relative incidence (subdistribution hazard ratio) and the rate (cause-specific hazard ratio) of recurrent ICH relative to the low-risk category, although this difference was not significant in either model. The CT SVD score had no significant effect on the subdistribution hazard ratio or cause-specific hazard ratio of recurrent ICH, although those with a CT SVD score of 1, 2 or 3 tended to have an increased hazard of death.

Table 10.20 Three year cumulative incidence of recurrent ICH and death in 91 LINCHPIN participants with first-ever SVD-associated lobar ICH

	Number of participants	Recurrent ICH			Death		
		Number of events	3 year cumulative incidence rate	p value	Number of events	3 year cumulative incidence rate	p value
Sex							
Female	58	10	17.9% (9.1-29.2)	0.472	18	33.8% (21.1-47.0)	0.668
Male	33	4	13.7% (4.1-29.0)		9	28.9% (14.0-45.6)	
CT SVD score							
0	38	5	14.3% (5.0-28.4)	0.630	7	20.9% (8.8-36.5)	0.053
1, 2, 3	53	9	17.8% (8.6-29.7)		20	39.6% (25.8-53.0)	
Edinburgh CT-APOE CAA criteria							
Low	22	1	4.5% (0.3-19.5)	0.063	8	38.6% (17.6-59.4)	0.146
Intermediate	33	4	13.1% (4.0-27.9)		13	41.0% (23.5-57.8)	
High	36	9	26.8% (12.7-43.2)		6	18.1% (7.0-33.5)	
Edinburgh CT-APOE CAA criteria							
Low	22	1	4.5% (0.3-19.5)	0.105	8	38.6% (17.6-59.4)	0.468
Intermediate/ High	69	13	20.4% (11.4-31.3)		19	29.7% (18.8-41.4)	

Data are % (95%CI). APOE = apolipoprotein E. CAA – cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. SVD = small vessel disease.

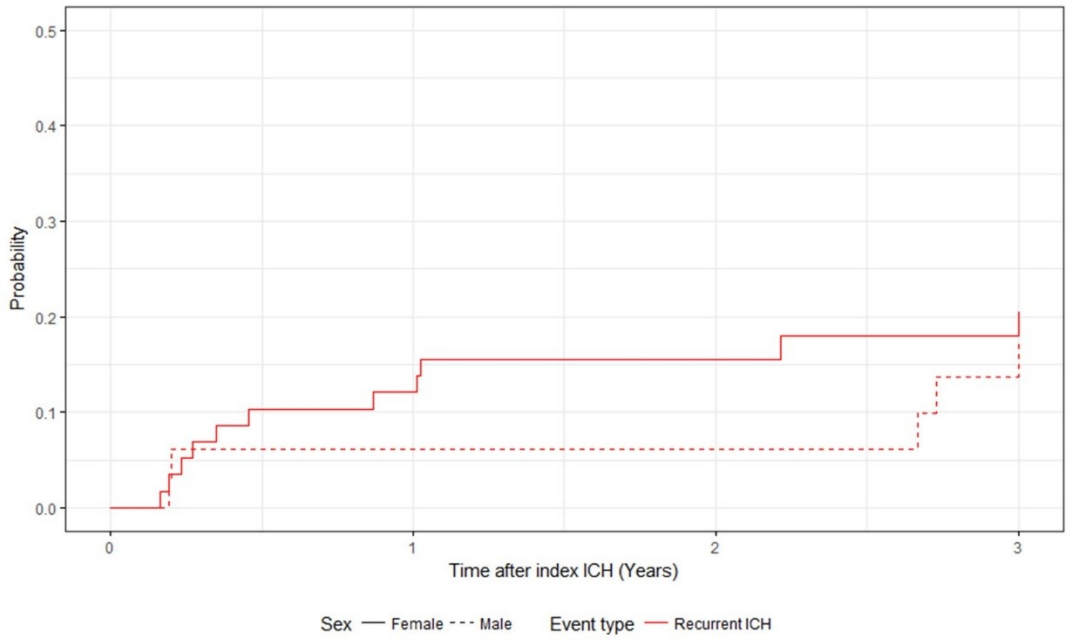
Figure 10.12 Cumulative incidence of recurrent ICH and death in first-ever SVD-associated lobar ICH in LINCHPIN

A & B. Recurrent ICH and death stratified by sex

C & D Recurrent ICH and death stratified by CT SVD score

E to H Recurrent ICH and death stratified by the Edinburgh CT-APOE CAA criteria

A



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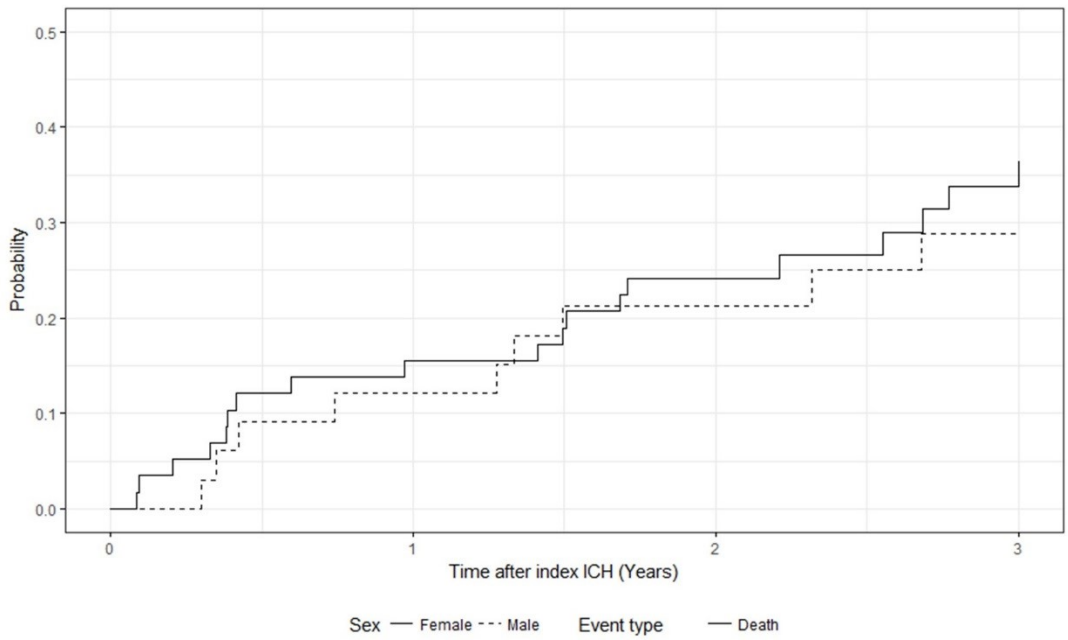

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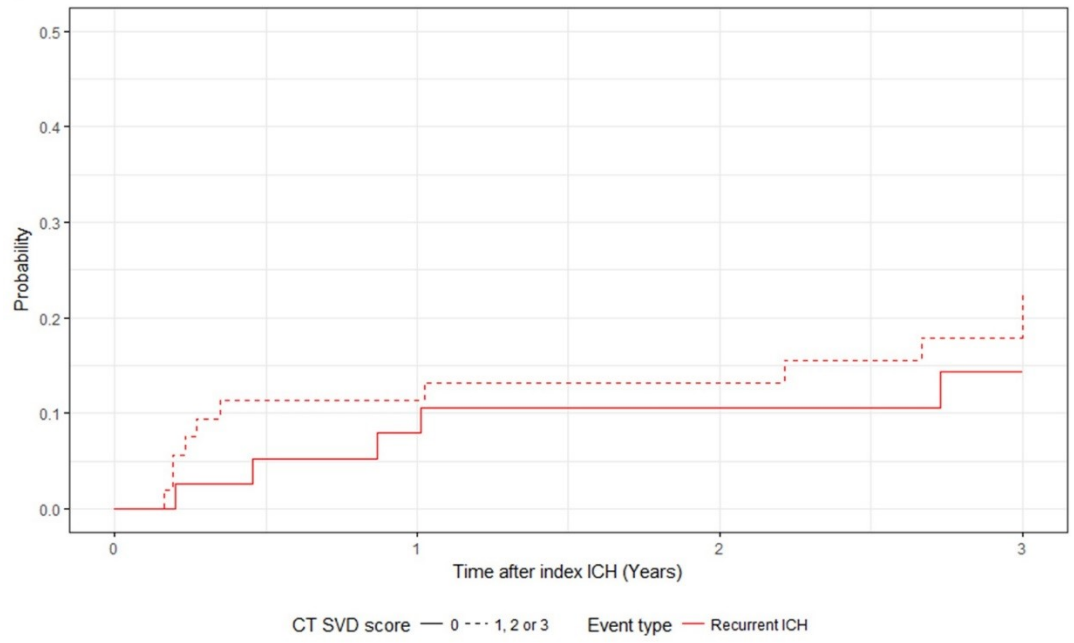
0.5
0.4
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0.2
0.1
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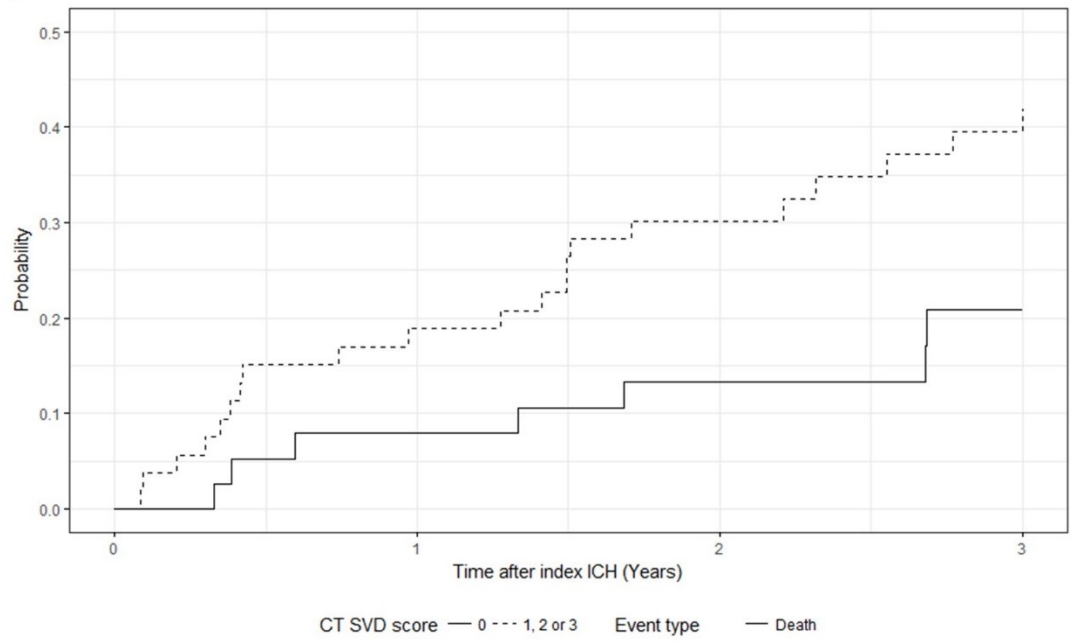
Probability
0
1
2
3
Time after index ICH (Years)
CT SVD score
0
1, 2 or 3
Event type
Recurrent ICH

D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Probability
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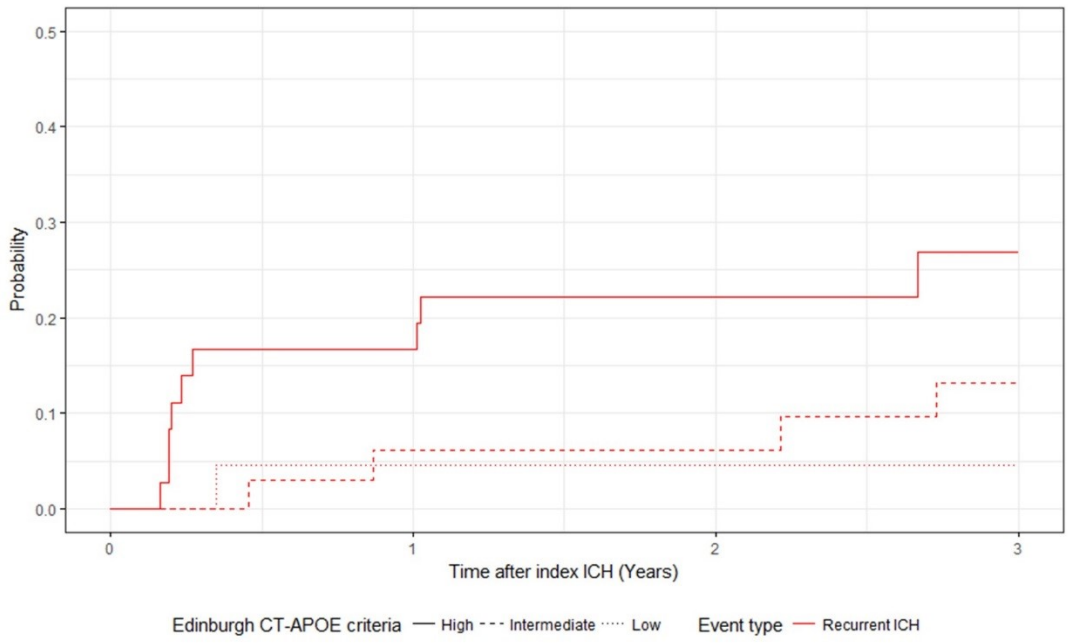
Time after index ICH (Years)
CT SVD score
0
1, 2 or 3
Event type
Death

E


0.5
0.4
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Probability
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1
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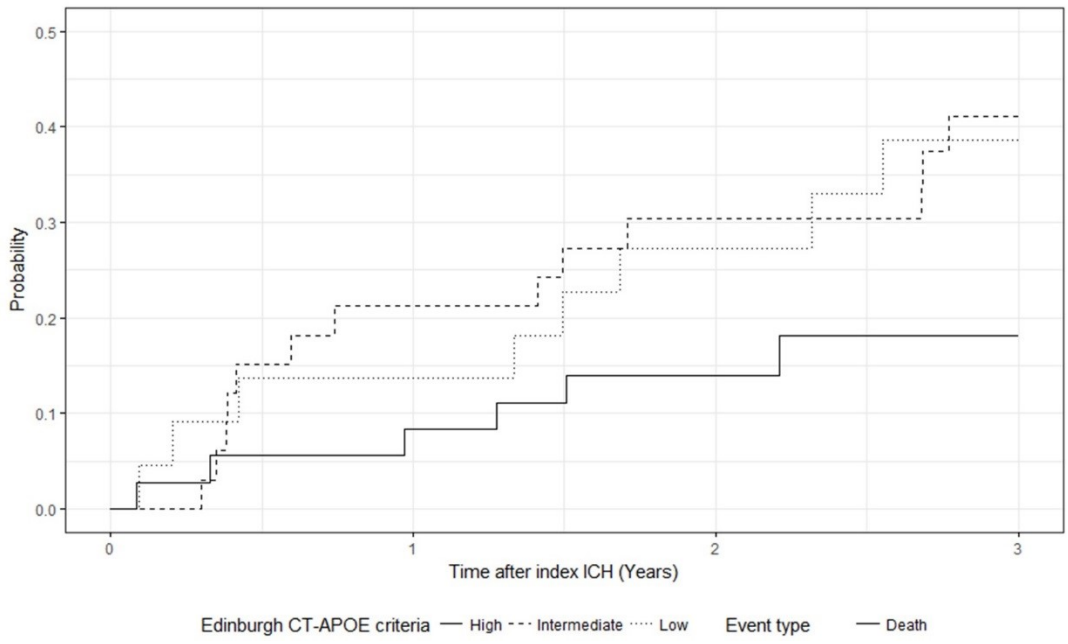
3
Time after index ICH (Years)
Edinburgh CT-APOE criteria
High
Intermediate
Low
Event type
Recurrent ICH

F


0.5
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Probability
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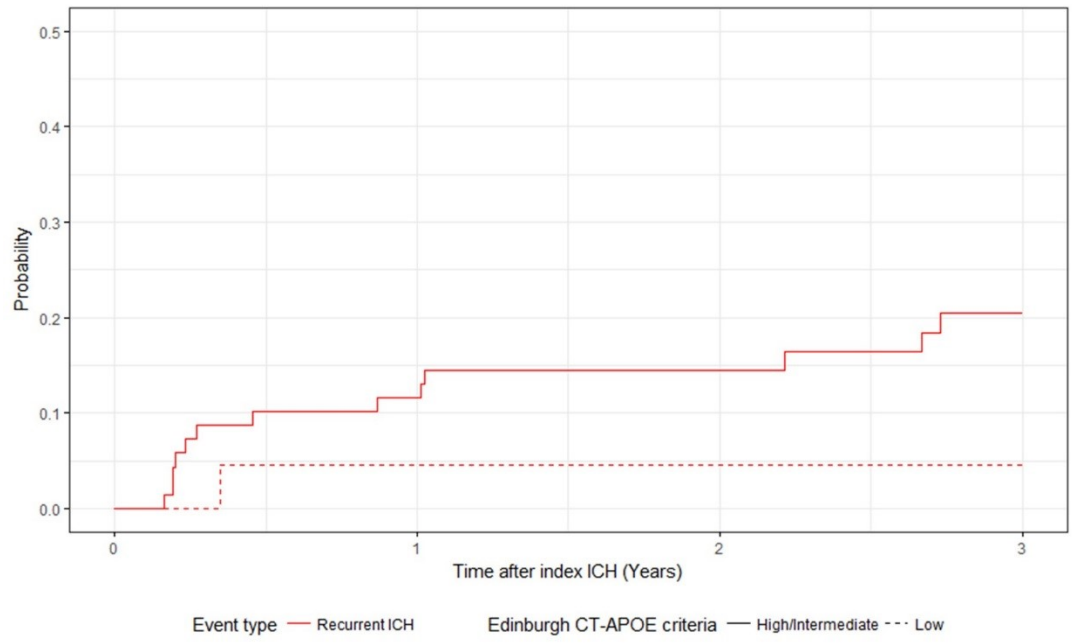
Time after index ICH (Years)
Edinburgh CT-APOE criteria
High
Intermediate
Low
Event type
Death

G



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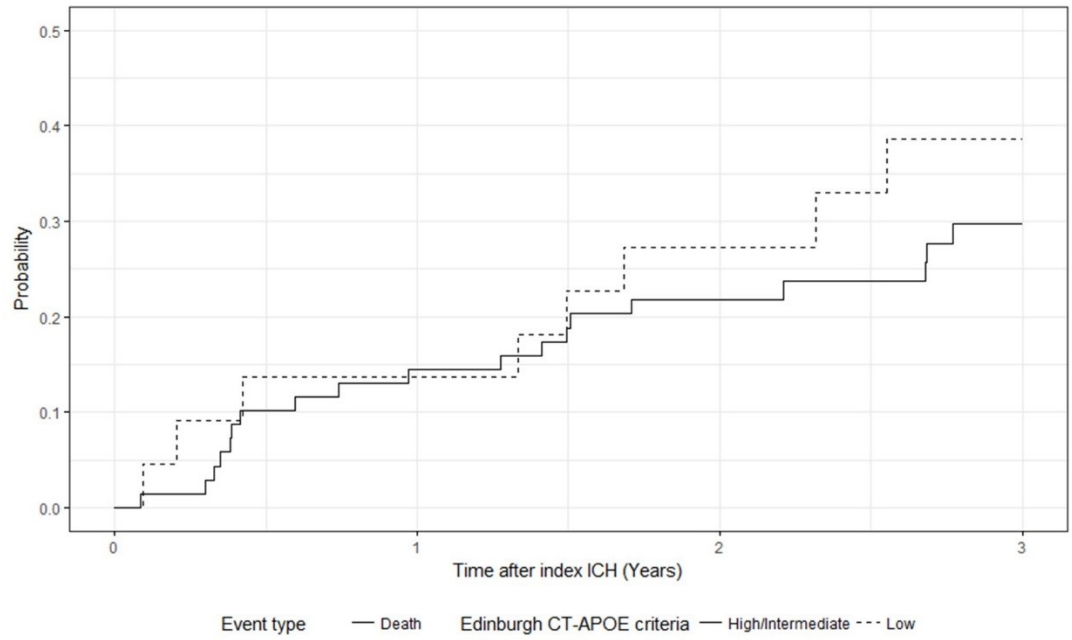


APOE = Apolipoprotein E. CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. SVD = small vessel disease.

Table 10.21 Univariable subdistribution and cause-specific hazard models for recurrent ICH and death in 91 LINCHPIN participants with first-ever SVD-associated lobar ICH

	Subdistribution hazard model						Cause-specific hazard model					
	Recurrent ICH			Death			Recurrent ICH			Death		
	Hazard ratio		p value	Hazard ratio		p value	Hazard ratio		p value	Hazard ratio		p value
Sex												
Female	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Male	0.66	(0.21-2.06)	0.476	0.84	(0.38-1.86)	0.670	0.62	(0.19-1.98)	0.417	0.77	(0.35-1.71)	0.521
CT SVD score												
0	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
1, 2, 3	1.32	(0.45-3.84)	0.615	2.29	(0.98-5.32)	0.055	1.51	(0.50-4.49)	0.464	2.48	(1.05-5.88)	0.039
Edinburgh CT-APOE CAA criteria												
Low	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Intermediate	2.66	(0.30-23.5)	0.379	1.08	(0.46-2.56)	0.860	2.61	(0.29-23.4)	0.391	1.10	(0.46-2.65)	0.834
High	6.56	(0.83-52.0)	0.075	0.43	(0.15-1.24)	0.119	6.17	(0.78-48.8)	0.084	0.53	(0.18-1.53)	0.242
Edinburgh CT-APOE CAA criteria												
Low	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Intermediate/high	4.51	(0.59-34.53)	0.147	0.73	(0.33-1.65)	0.454	4.34	(0.57-33.2)	0.157	0.82	(0.36-1.88)	0.641

Data are hazard ratio (95%CI). APOE = apolipoprotein E. CAA – cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. SVD = small vessel disease.

The Edinburgh CT-APOE variable did not obey the proportional hazard assumption; therefore, I did not perform multivariable analyses in the LINCHPIN cohort.

CROMIS-2-DNA cohort

Twenty-four of the 293 CROMIS-2-DNA participants with first-ever SVD-associated lobar ICH had a recurrent ICH while 51 died during follow up.

The 3-year cumulative incidence rate for recurrent ICH and death according to sex, CT SVD score and Edinburgh CT-only diagnostic criteria are shown in Table 10.22 and Figure 10.13.

The 3-year cumulative incidence rate for recurrent ICH was significantly higher in those with CT SVD score of 1, 2 or 3 compared with a CT SVD score of 0 (13.6%, 95%CI 8.1-20.6 versus 5.2%, 95%CI 2.4-9.6, p=0.011). The 3-year cumulative incidence of death was also significantly higher in those with a CT SVD score of 1, 2 or 3. There was no significant change in the 3-year cumulative incidence of recurrent ICH with the Edinburgh CT-APOE diagnostic criteria.

The univariable subdistribution and cause-specific hazard models for sex, CT SVD score and Edinburgh CT-only diagnostic criteria are shown in Table 10.23. CT SVD score of 1, 2 or 3 significantly increased the relative incidence (subdistribution hazard ratio) and the rate (cause-specific hazard ratio) of recurrent ICH and death. The Edinburgh CT-APOE criteria had no significant effect on the subdistribution hazard ratio or cause-specific hazard ratio of recurrent ICH.

In multivariable models adjusting for CT SVD score and Edinburgh CT-APOE criteria (Table 10.24), CT SVD score of 1, 2 or 3 was associated with an increased risk and rate of recurrent ICH. The Edinburgh CT-APOE criteria had no significant effect on the subdistribution hazard ratio or cause-specific hazard ratio of recurrent ICH or death.

I performed a secondary multivariable analysis by pooling the LINCHPIN and CROMIS-2-DNA data (384 participants with first-ever SVD-associated lobar ICH) and then fitting multivariable models (Table 10.25). High risk on the Edinburgh CT-APOE diagnostic criteria was associated with increased risk of recurrent ICH (subdistribution hazard model), and borderline significantly increased rate of recurrent ICH (cause-specific hazard model). CT SVD score of 1, 2 or 3 had a significant association with recurrent ICH and death in both the subdistribution and cause-specific hazard models.

Table 10.22 Three year cumulative incidence of recurrent ICH and death in 293 CROMIS-2-DNA participants with first-ever SVD-associated lobar ICH

	Number of participants	Recurrent ICH			Death		
		Number of events	3 year cumulative incidence rate	p value	Number of events	3 year cumulative incidence rate	p value
Sex							
Female	134	13	10.5% (5.8-16.7)	0.333	28	24.3% (16.7-32.6)	0.131
Male	159	11	7.4% (3.9-12.3)		23	15.0% (9.9-21.2)	
CT SVD score							
0	169	8	5.2% (2.4-9.6)	0.011	22	13.7% (8.9-19.6)	0.025
1, 2, 3	124	16	13.6% (8.1-20.6)		29	26.4% (18.4-35.1)	
Edinburgh CT-APOE CAA criteria							
Low	110	8	7.9% (3.6-14.4)	0.747	19	18.6% (11.7-26.9)	0.958
Intermediate	116	9	8.1% (3.9-14.1)		21	19.9% (12.8-28.1)	
High	67	7	11.6% (5.0-21.3)		11	18.2% (9.6-29.1)	
Edinburgh CT-APOE CAA criteria							
Low	110	8	7.9% (3.6-14.4)	0.657	19	18.6% (11.7-26.9)	0.941
Intermediate/high	183	16	9.3% (5.5-14.3)		32	19.2% (13.6-25.7)	

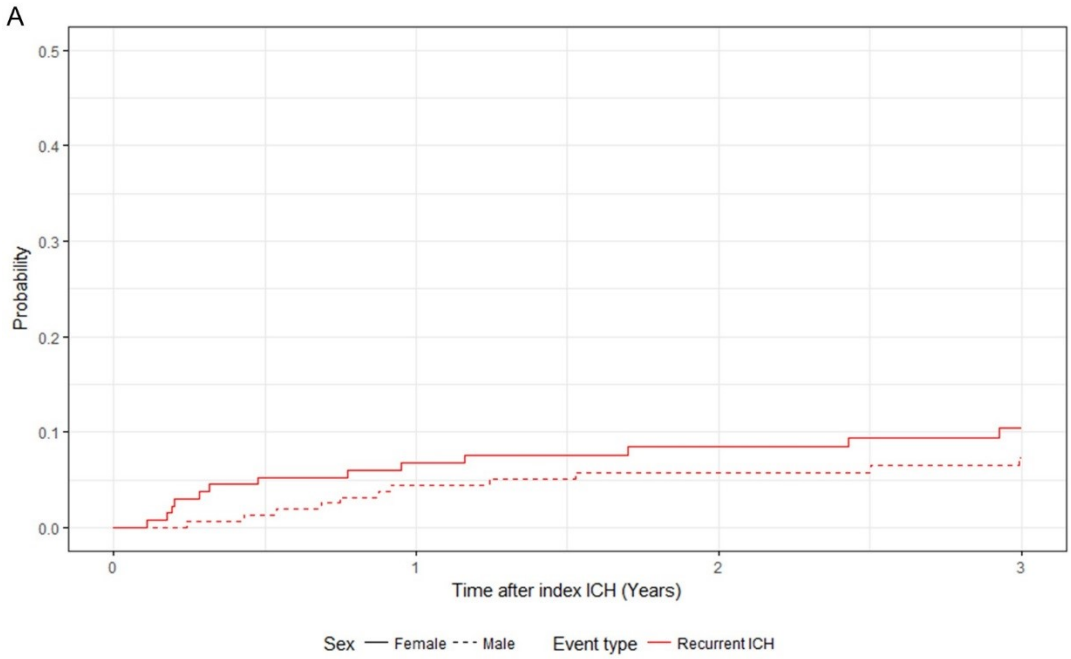
Data are % (95%CI). APOE = apolipoprotein E. CAA – cerebral amyloid angiopathy. CROMIS = clinical relevance of microbleeds in stroke. CT = computed tomography. DNA = deoxyribonucleic acid. ICH = intracerebral haemorrhage. SVD = small vessel disease.

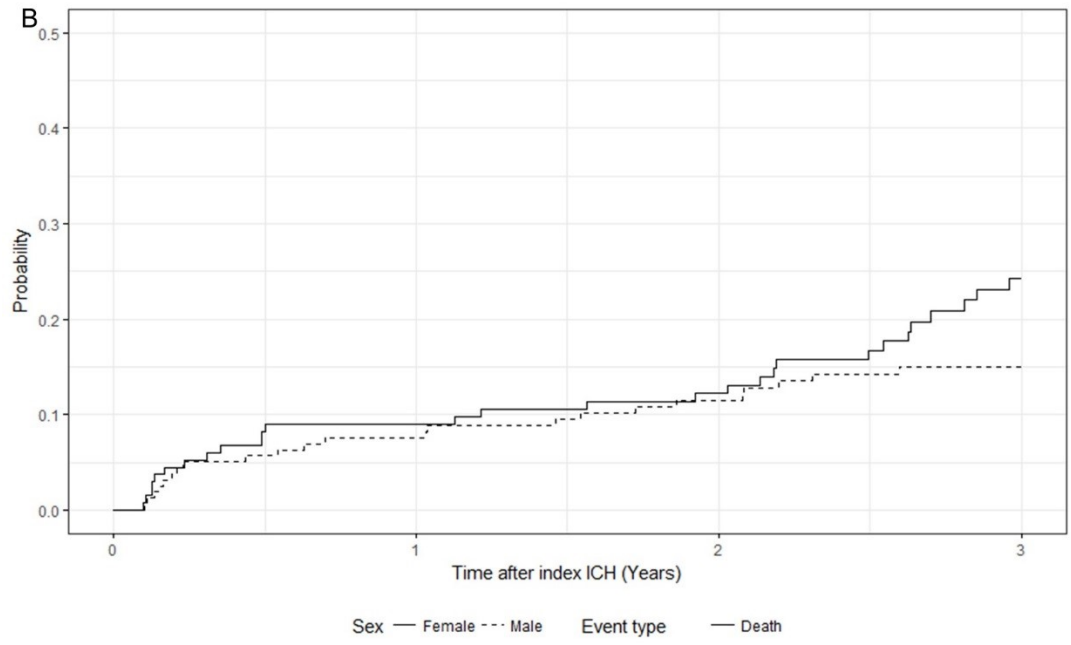
Figure 10.13 Cumulative incidence of recurrent ICH and death in first-ever SVD-associated lobar ICH in CROMIS-2-DNA

A & B. Recurrent ICH and death stratified by sex

C & D Recurrent ICH and death stratified by CT SVD score

E to H Recurrent ICH and death stratified by the Edinburgh CT-APOE CAA criteria





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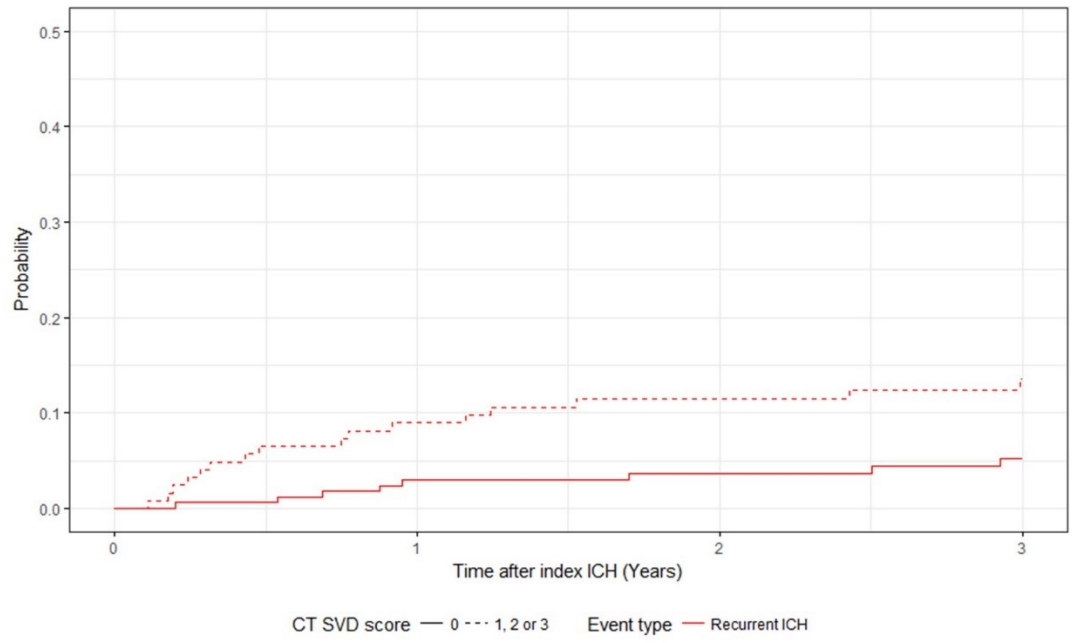
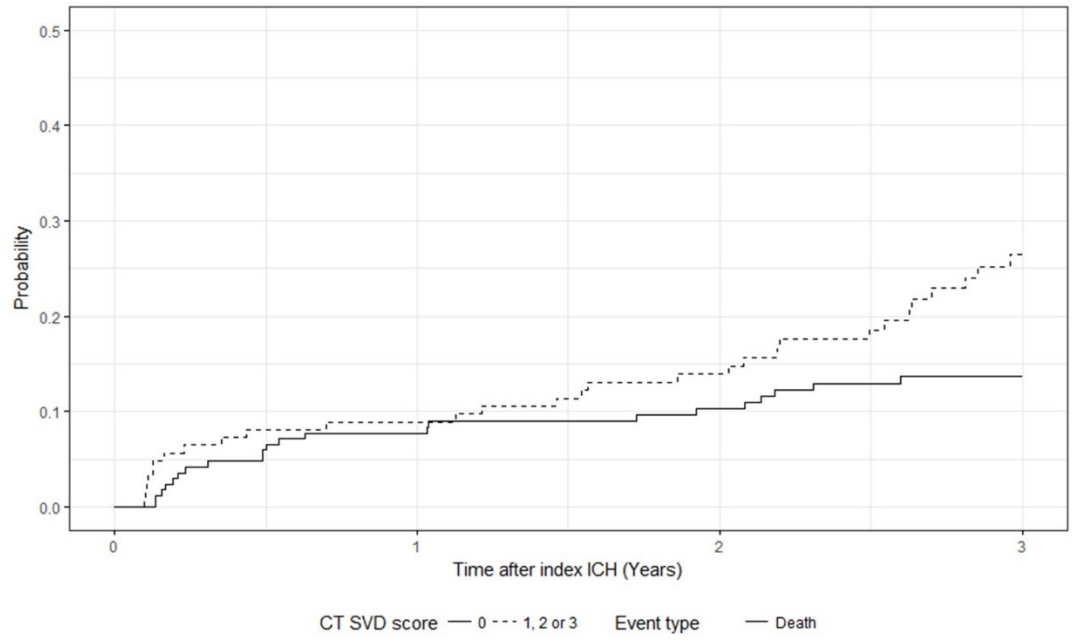

0.5
0.4
0.3
0.2
0.1
0.0
Probability
0
1
2
3
Time after index ICH (Years)
CT SVD score
0
1, 2 or 3
Event type
Recurrent ICH

D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Probability
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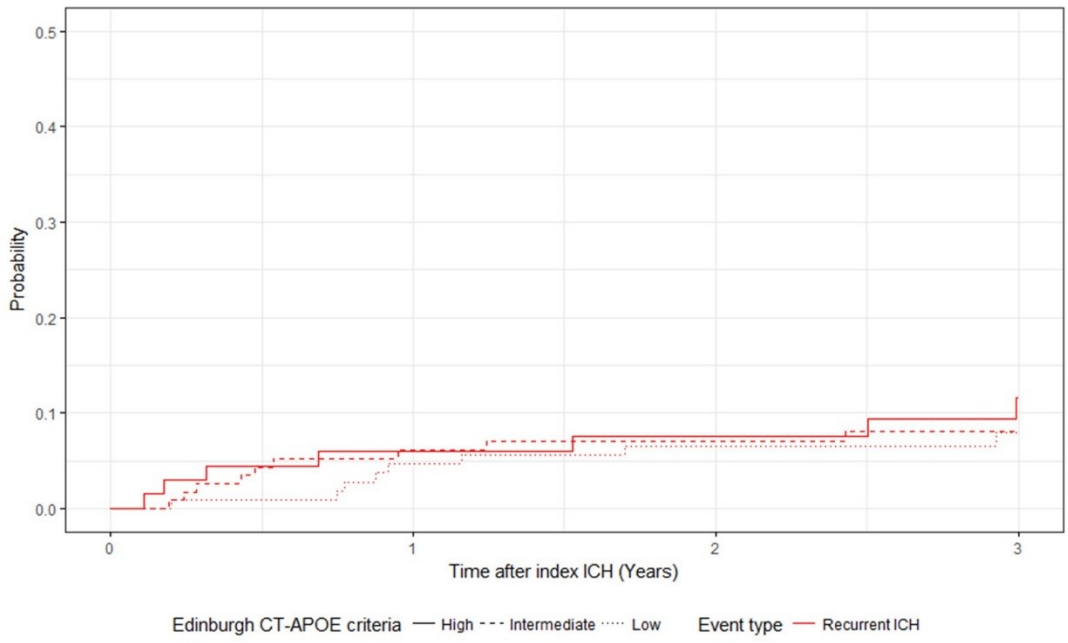
Time after index ICH (Years)
CT SVD score
0
1, 2 or 3
Event type
Death

E


0.5
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0.2
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Probability
0
1
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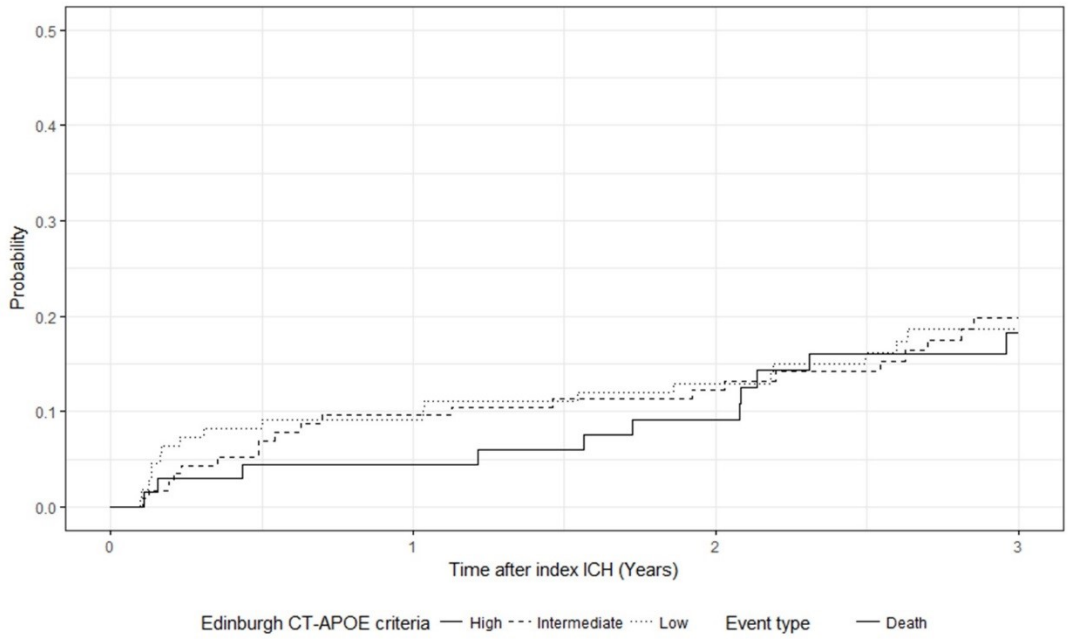
Time after index ICH (Years)
Edinburgh CT-APOE criteria
High
Intermediate
Low
Event type
Recurrent ICH

F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Probability
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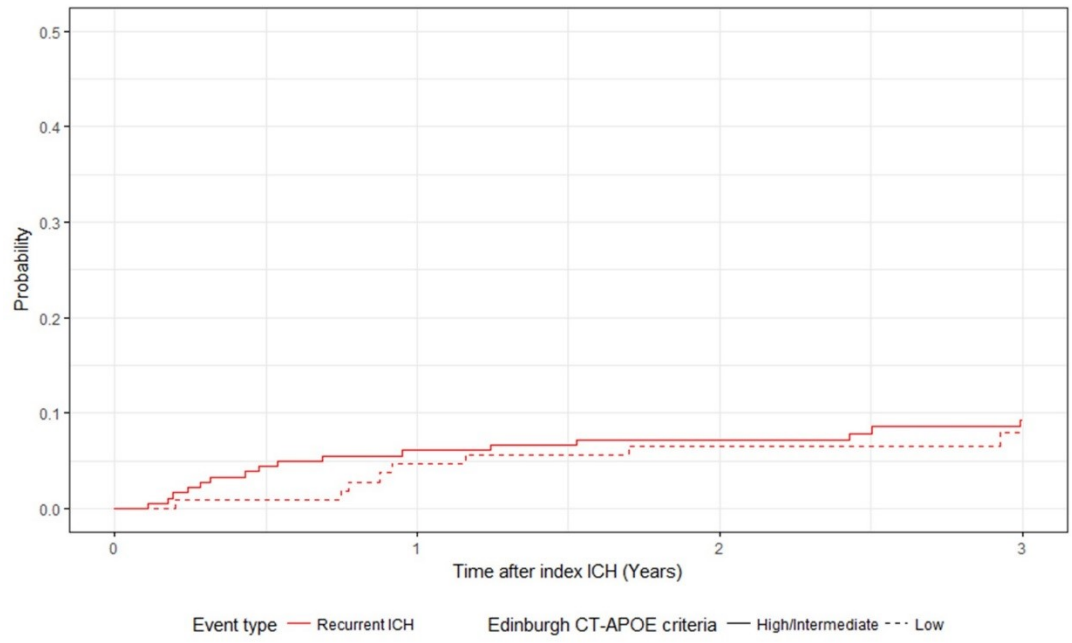
Time after index ICH (Years)
Edinburgh CT-APOE criteria
High
Intermediate
Low
Event type
Death

G



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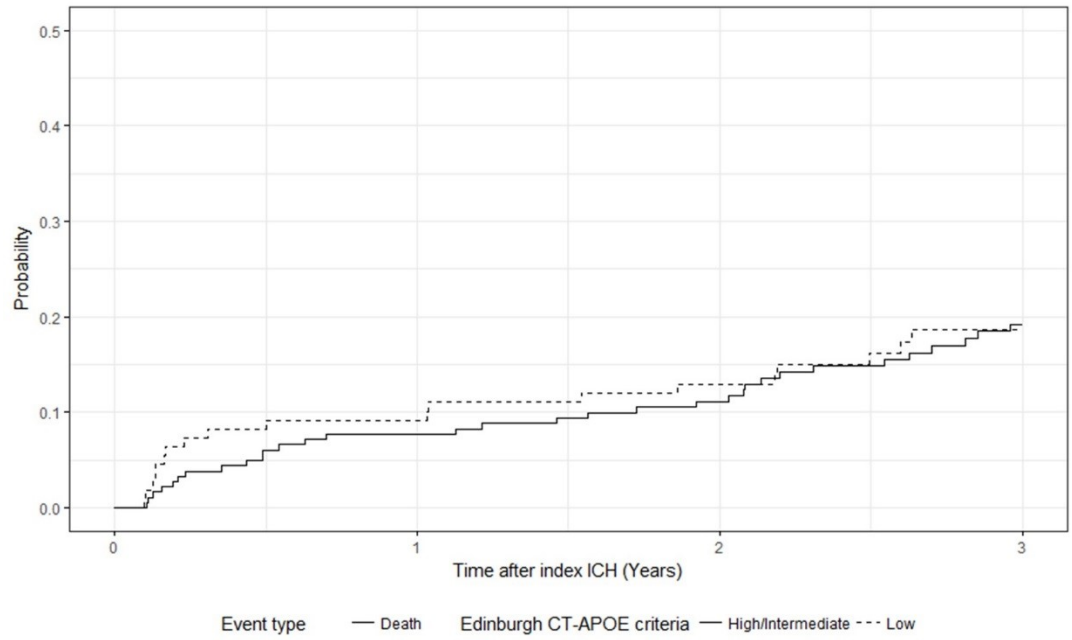


APOE = apolipoprotein E. CAA = cerebral amyloid angiopathy. CROMIS = clinical relevance of microbleeds in stroke. CT = computed tomography. ICH = intracerebral haemorrhage. SVD = small vessel disease.

Table 10.23 Univariable subdistribution and cause-specific hazard models for recurrent ICH and death in 293 CROMIS-2-DNA participants with first-ever SVD-associated lobar ICH

	Subdistribution hazard model						Cause-specific hazard model					
	Recurrent ICH			Death			Recurrent ICH			Death		
	Hazard ratio		p value	Hazard ratio		p value	Hazard ratio		p value	Hazard ratio		p value
Sex												
Female	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Male	0.68	(0.30-1.50)	0.336	0.66	(0.38-1.14)	0.136	0.66	(0.29-1.46)	0.303	0.64	(0.37-1.10)	0.108
CT SVD score												
0	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
1, 2, 3	2.87	(1.24-6.67)	0.014	1.87	(1.08-3.26)	0.026	3.01	(1.29-7.03)	0.011	2.00	(1.15-3.49)	0.014
Edinburgh CT-APOE CAA criteria												
Low	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Intermediate	1.07	(0.42-2.78)	0.882	1.02	(0.55-1.89)	0.957	1.06	(0.41-2.75)	0.906	1.03	(0.55-1.91)	0.929
High	1.45	(0.53-3.98)	0.466	0.91	(0.44-1.91)	0.812	1.40	(0.51-3.85)	0.520	0.93	(0.44-1.96	0.853
Edinburgh CT-APOE CAA criteria												
Low	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Intermediate/high	1.21	(0.52-2.82)	0.653	0.98	(0.56-1.73)	0.943	1.18	(0.51-2.77)	0.697	0.99	(0.56-1.75)	0.982

Data are hazard ratio (95%CI). APOE = apolipoprotein E. CAA – cerebral amyloid angiopathy. CROMIS = clinical relevance of microbleeds in stroke. CT = computed tomography. DNA = deoxyribonucleic acid. ICH = intracerebral haemorrhage. SVD = small vessel disease.

Table 10.24 Multivariable subdistribution and cause-specific hazard models for recurrent ICH and death in 293 CROMIS-2-DNA participants with first-ever SVD-associated lobar ICH

	Subdistribution hazard model						Cause-specific hazard model					
	Recurrent ICH			Death			Recurrent ICH			Death		
	Hazard ratio		p value	Hazard ratio		p value	Hazard ratio		p value	Hazard ratio		p value
CT SVD score												
0	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
1, 2, 3	2.84	(1.23-6.56)	0.015	1.89	(1.08-3.29)	0.026	2.97	(1.27-6.96)	0.012	2.01	(1.16-3.51)	0.014
Edinburgh CT-APOE CAA criteria												
Low	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Intermediate	1.04	(0.40-2.70)	0.930	0.98	(0.53-1.81)	0.946	1.02	(0.39-2.64)	0.974	0.99	(0.53-1.85)	0.984
High	1.36	(0.50-3.72)	0.548	0.86	(0.41-1.81)	0.696	1.29	(0.47-3.57)	0.623	0.88	(0.42-1.85)	0.739

Data are hazard ratio (95%CI). APOE = apolipoprotein E. CAA – cerebral amyloid angiopathy. CROMIS = clinical relevance of microbleeds in stroke. CT = computed tomography. DNA = deoxyribonucleic acid. ICH = intracerebral haemorrhage. SVD = small vessel disease.

Table 10.25 Secondary analysis: Multivariable subdistribution and cause-specific hazard models for recurrent ICH and death in 384 LINCHPIN and CROMIS-2-DNA participants with first-ever SVD-associated lobar ICH

	Subdistribution hazard model						Cause-specific hazard model					
	Recurrent ICH			Death			Recurrent ICH			Death		
	Hazard ratio		p value	Hazard ratio		p value	Hazard ratio		p value	Hazard ratio		p value
CT SVD score												
0	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
1, 2, 3	2.32	(1.19-4.51)	0.013	2.12	(1.34-3.36)	0.001	2.50	(1.28-4.88)	0.008	2.27	(1.44-3.60)	<0.001
Edinburgh CT-APOE CAA criteria												
Low	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Intermediate	1.25	(0.54-2.92)	0.605	1.06	(0.64-1.75)	0.822	1.22	(0.52-2.87)	0.641	1.07	(0.65-1.78)	0.785
High	2.35	(1.05-5.30)	0.039	0.74	(0.40-1.36)	0.329	2.24	(0.99-5.07)	0.053	0.81	(0.44-1.48)	0.492

Data are hazard ratio (95%CI). APOE = apolipoprotein E. CAA – cerebral amyloid angiopathy. CROMIS = clinical relevance of microbleeds in stroke. CT = computed tomography. DNA = deoxyribonucleic acid. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. SVD = small vessel disease.

Secondary analyses

I performed pre-specified secondary analyses assessing the effect of APOE ε2 and APOE ε4 allele possession on the risk of recurrent ICH or death during follow up in 384 LINCHPIN and CROMIS-2-DNA participants with SVD-associated lobar ICH. Thirty-eight of the participants had a recurrent ICH, and 78 died during follow up.

The 3-year cumulative incidence of recurrent ICH was higher in those who possessed an APOE ε2 allele, although this was borderline significant (Figure 10.14 and Table 10.26). There was no significant difference in the cumulative incidence of recurrent ICH between those with and without an APOE ε4 allele.

Multivariable models adjusting for history of previous ICH, antihypertensive drug use at hospital discharge, CT SVD score and the Edinburgh CT-APOE diagnostic criteria and APOE ε2 allele possession are shown in Table 10.27. CT SVD score 1, 2 or 3 and APOE ε2 allele possession were associated with an increased risk (subdistribution hazard model) and rate (cause-specific hazard model) of recurrent ICH. High risk on the Edinburgh CT-APOE criteria was associated with a borderline significant increased risk and rate of recurrent ICH.

Finally, I refitted the above models using subarachnoid haemorrhage, finger-like projections and APOE ε4 allele possession in place of the Edinburgh CT-APOE criteria. In these models (Table 10.28), only CT SVD score 1, 2 or 3 and APOE ε2 allele possession were associated with an increased risk (subdistribution hazard model) and rate (cause-specific hazard model) of recurrent ICH.

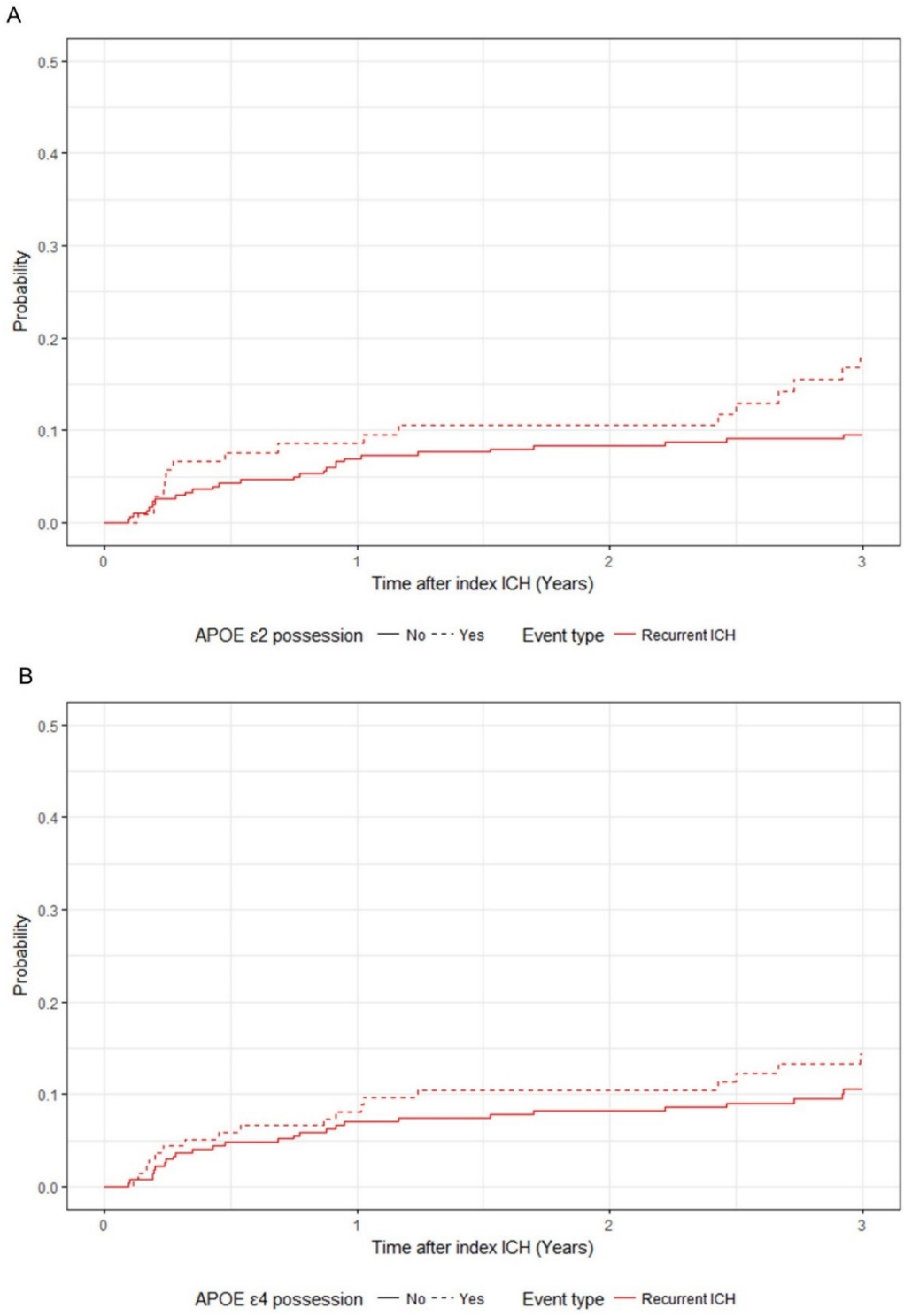
10.4.2.5 Confounders

The proportion of first-ever SVD-associated lobar ICH LINCHPIN participants on an antiplatelet, anticoagulant or antihypertensive drug at hospital discharge was similar between those with and without a recurrent ICH (Table 10.29), as well as in the Edinburgh CT-APOE risk categories (Table 10.30).

In CROMIS-2 DNA, the proportion of participants with a first-ever SVD-associated lobar ICH on an antihypertensive drug at hospital discharge was lower in those who had a recurrent ICH (57%) compared to those without a recurrent ICH (72%) (Table 10.31), and lower in those classified as high risk on the Edinburgh CT-only criteria (67%) compared to low risk (71%) (Table 10.32). The proportion taking an antiplatelet or anticoagulant drug at hospital discharge was similar across the groups.

Figure 10.14 Cumulative incidence of recurrent ICH in SVD-associated lobar ICH in the pooled LINCHPIN and CROMIS-2-DNA data

A. APOE ε2. B. APOE ε4.



APOE = apolipoprotein E. CROMIS = clinical relevance of microbleeds in stroke. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. SVD = small vessel disease.

Table 10.26 Secondary analysis: Three year cumulative incidence of recurrent ICH and death in 410 LINCHPIN and CROMIS-2-DNA participants with SVD-associated lobar ICH

	Number of participants	Recurrent ICH			Death		
		Number of events	3 year cumulative incidence rate	p value	Number of events	3 year cumulative incidence rate	p value
APOE ε2 allele possession							
No	304	28	9.6% (6.5-13.3)	0.053	66	23.6% (18.8-28.9)	0.486
Yes	106	17	18.1% (11.0-26.7)		19	19.5% (12.2-28.0)	
APOE ε4 allele possession							
No	273	27	10.5% (7.1-14.7)	0.297	62	24.2% (19.1-29.7)	0.162
Yes	137	18	14.4% (8.8-21.2)		23	19.4% (12.7-27.1)	

Data are % (95%). APOE = apolipoprotein E. CROMIS = clinical relevance of microbleeds in stroke. DNA = deoxyribonucleic acid. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. SVD = small vessel disease.

Table 10.27 Secondary analysis: Multivariable subdistribution and cause-specific hazard models for recurrent ICH and death in 381 LINCHPIN and CROMIS-2-DNA participants with SVD-associated lobar ICH

	Subdistribution hazard model						Cause-specific hazard model					
	Recurrent ICH			Death			Recurrent ICH			Death		
	Hazard ratio		p value	Hazard ratio		p value	Hazard ratio		p value	Hazard ratio		p value
History of ICH												
No	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Yes	1.82	(0.57-5.83)	0.314	1.29	(0.47-3.59)	0.623	1.86	(0.57-6.14)	0.307	1.25	(0.45-3.47)	0.664
Antihypertensive on discharge												
No	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Yes	0.64	(0.34-1.21)	0.170	0.96	(0.57-1.61)	0.862	0.61	(0.31-1.18)	0.140	0.93	(0.57-1.53)	0.781
CT SVD score												
0	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
1, 2, 3	2.09	(1.06-4.15)	0.034	2.31	(1.43-3.73)	0.001	2.29	(1.17-4.49)	0.016	2.44	(1.52-3.94)	<0.001
Edinburgh CT-APOE CAA criteria												
Low	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Intermediate	1.22	(0.53-2.81)	0.639	1.03	(0.62-1.72)	0.911	1.22	(0.53-2.80)	0.637	1.04	(0.63-1.74)	0.869
High	2.22	(0.99-4.97)	0.054	0.71	(0.39-1.31)	0.276	2.12	(0.95-4.74)	0.068	0.76	(0.41-1.42)	0.395
APOE ε2 allele possession												
No	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Yes	2.17	(1.13-4.15)	0.019	0.78	(0.42-1.45)	0.428	2.07	(1.06-4.03)	0.032	0.81	(0.46-1.44)	0.476

Data are hazard ratio (95%CI). APOE = apolipoprotein E. CAA – cerebral amyloid angiopathy. CROMIS = clinical relevance of microbleeds in stroke. CT = computed tomography. DNA = deoxyribonucleic acid. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. SVD = small vessel disease.

Table 10.28 Secondary analysis: Multivariable subdistribution and cause-specific hazard models for recurrent ICH and death in 381 LINCHPIN and CROMIS-2-DNA participants with SVD-associated lobar ICH

	Subdistribution hazard model						Cause-specific hazard model					
	Recurrent ICH			Death			Recurrent ICH			Death		
	Hazard ratio		p value	Hazard ratio		p value	Hazard ratio		p value	Hazard ratio		p value
History of ICH												
No	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Yes	1.92	(0.59-6.19)	0.277	1.27	(0.47-3.42)	0.642	1.93	(0.58-6.39)	0.282	1.22	(0.44-3.38)	0.703
Antihypertensive on discharge												
No	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Yes	0.63	(0.33-1.21)	0.167	0.99	(0.59-1.66)	0.968	0.60	(0.31-1.17)	0.137	0.98	(0.59-1.61)	0.929
CT SVD score												
0	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
1, 2, 3	2.10	(1.05-4.21)	0.037	2.31	(1.43-3.73)	0.001	2.29	(1.17-4.51)	0.016	2.47	(1.53-3.98)	<0.001
Subarachnoid haemorrhage												
No	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Yes	1.51	(0.79-2.89)	0.216	1.04	(0.65-1.69)	0.860	1.48	(0.75-2.91)	0.254	1.07	(0.67-1.72)	0.772
Finger-like projections												
No	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Yes	1.47	(0.70-3.07)	0.308	0.98	(0.54-1.77)	0.933	1.47	(0.70-3.09)	0.309	1.05	(0.58-1.90)	0.867
APOE ε2 allele possession												
No	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Yes	2.16	(1.13-4.15)	0.020	0.75	(0.40-1.38)	0.349	2.08	(1.07-4.04)	0.031	0.78	(0.44-1.38)	0.395
APOE ε4 allele possession												
No	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--	1.00	(Reference)	--
Yes	1.50	(0.75-2.98)	0.249	0.62	(0.37-1.04)	0.072	1.44	(0.74-2.80)	0.285	0.63	(0.37-1.06)	0.082

Data are hazard ratio (95%CI). APOE = apolipoprotein E. CAA – cerebral amyloid angiopathy. CROMIS = clinical relevance of microbleeds in stroke. CT = computed tomography. DNA = deoxyribonucleic acid. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome. SVD = small vessel disease.

Table 10.29 Medication use at hospital discharge in LINCHPIN participants with first-ever lobar ICH stratified by the outcome of recurrent ICH during follow-up

	No recurrent ICH (n=77)	Recurrent ICH (n=14)
Antiplatelet at discharge*	2 (3%)	0 (0%)
Anticoagulant at discharge*	2 (3%)	1 (8%)
Antihypertensive at discharge*	42 (56%)	6 (50%)

* Data is not available for four LINCHPIN participants with first-ever lobar ICH who survived more than 30 days but died before hospital discharge. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome.

Table 10.30 Medication use at hospital discharge in LINCHPIN participants with first-ever lobar ICH stratified by Edinburgh CT-APOE criteria classification

	Low risk CT-APOE Edinburgh CAA criteria (n=22)	Intermediate risk CT-APOE Edinburgh CAA criteria (n=33)	High risk CT-APOE Edinburgh CAA criteria (n=36)
Antiplatelet at discharge*	1 (5%)	0 (0%)	1 (3%)
Anticoagulant at discharge*	0 (0%)	2 (3%)	2 (6%)
Antihypertensive at discharge*	12 (57%)	14 (42%)	22 (67%)

* Data is not available for four LINCHPIN participants with first-ever lobar ICH who survived more than 30 days but died before hospital discharge. APOE = apolipoprotein E. CAA = cerebral amyloid angiopathy. CT = computed tomography. ICH = intracerebral haemorrhage. LINCHPIN = Lothian intracerebral haemorrhage pathology, imaging and neurological outcome.

Table 10.31 Medication use at hospital discharge in CROMIS-2-DNA participants with first-ever lobar ICH stratified by the outcome of recurrent ICH during follow-up

	No recurrent ICH (n=269)	Recurrent ICH (n=24)
Antiplatelet at discharge*	10 (4%)	2 (8%)
Anticoagulant at discharge†	12 (5%)	0 (0%)
Antihypertensive at discharge†	186 (72%)	13 (57%)

* Data is not available for 12 CROMIS-2-DNA participants. † Data is not available for 13 CROMIS-2-DNA participants. CROMIS = clinical relevance of microbleeds in stroke. DNA = deoxyribonucleic acid. ICH = intracerebral haemorrhage.

Table 10.32 Medication use at hospital discharge in CROMIS-2-DNA participants with first-ever lobar ICH stratified by Edinburgh CT-APOE criteria classification

	Low risk CT-APOE Edinburgh CAA criteria (n=110)	Intermediate risk CT-APOE Edinburgh CAA criteria (n=116)	High risk CT-APOE Edinburgh CAA criteria (n=67)
Antiplatelet at discharge*	5 (5%)	3 (3%)	4 (6%)
Anticoagulant at discharge†	6 (6%)	4 (4%)	2 (3%)
Antihypertensive at discharge†	76 (71%)	81 (74%)	42 (67%)

* Data is not available for 12 CROMIS-2-DNA participants. † Data is not available for 13 CROMIS-2-DNA participants. APOE = apolipoprotein E. CAA = cerebral amyloid angiopathy. CROMIS = clinical relevance of microbleeds in stroke. CT = computed tomography. DNA = deoxyribonucleic acid. ICH = intracerebral haemorrhage.

10.5 Discussion

10.5.1 Main findings

- The relative risk and rate of recurrent ICH were significantly higher in participants with first-ever lobar ICH compared with non-lobar ICH in both the LATCH and CROMIS-2 cohorts.
- Edinburgh CT-only diagnostic criteria for CAA-associated lobar ICH
    - In multivariable models adjusting for CT SVD score and Edinburgh CT-only criteria in first-ever lobar ICH LATCH participants, high risk on the Edinburgh CT-only criteria was significantly associated with an increased relative risk and rate of recurrent ICH compared with the low-risk category.
    - In multivariable models adjusting for CT SVD score and Edinburgh CT-only criteria in first-ever lobar ICH CROMIS-2 participants, the relative risk and rate of recurrent ICH were higher in participants classified as high risk on the Edinburgh CT-only criteria compared with low risk, although this did not reach significance. CT SVD score of 1, 2 or 3 was associated with an increased risk and rate of both recurrent ICH and death.
    - In a two-step random-effects meta-analysis of the subdistribution hazard models of the LATCH and CROMIS-2 cohorts, participants classified as high risk on the Edinburgh CT-only criteria had a significantly higher subdistribution hazard ratio for recurrent ICH compared with the low-risk group. CT SVD score of 1, 2 or 3 was not associated with an increased subdistribution hazard ratio of recurrent ICH.
    - Secondary multivariable analyses assessing the individual components of the Edinburgh CT-only criteria showed that subarachnoid haemorrhage was associated with an increased relative risk and rate of recurrent ICH, while finger-like projections were associated with a non-significant increased risk of recurrent ICH.
- Edinburgh CT-APOE diagnostic criteria for CAA-associated lobar ICH

- In multivariable models adjusting for CT SVD score and Edinburgh CT- APOE criteria in the CROMIS-2-DNA cohort, CT SVD score of 1, 2 or 3 was associated with an increased risk and rate of recurrent ICH and death. The Edinburgh CT-APOE criteria were not associated with a statistically significantly increased risk or rate of recurrent ICH.
- The Edinburgh CT-APOE variable did not obey the proportional hazard assumption in the LINCHPIN cohort; therefore, I did not perform multivariable analyses in the LINCHPIN cohort.
- In a secondary meta-analysis, pooling the LINCHPIN and CROMIS-2-DNA cohorts, participants classified as high risk on the Edinburgh CT-APOE criteria had significantly higher risk and rate of recurrent ICH compared with the low-risk group. CT SVD score of 1, 2 or 3 was also associated with an increased hazard of recurrent ICH and death.
- Secondary multivariable analyses assessing the individual components of the Edinburgh CT-APOE criteria and APOE ε2 genotype showed that APOE ε2 allele possession was independently associated with an increased risk and rate of recurrent ICH. Subarachnoid haemorrhage, finger-like projections and APOE ε4 allele possession were not significantly associated with an increased risk of recurrent ICH.

10.5.2 Strengths of the studies

I used data from three prospective cohort studies of ICH. The LATCH and LINCHPIN studies are overlapping community-based cohort studies in the Lothian Health Board region of Scotland. The Edinburgh criteria were developed in the LINCHPIN study. The CROMIS-2 study is a large multicentre UK-based cohort study of ICH, which provided the opportunity to evaluate the prognostic value of the Edinburgh criteria in an external cohort. There were few missing baseline data. To minimise information bias, we rated the diagnostic non-contrast brain CT scans using a standardised pro forma, masked to baseline data and outcome information. All studies had

comprehensive follow-up for recurrent ICH and death, with >95% completeness of follow up.

I used appropriate statistical approaches to analyse the data. A competing event is one whose occurrence precludes the occurrence of the primary event.[349] When studying recurrent ICH as the primary outcome, death is a major competing event. Traditional approaches to time-to-event analysis, such as the Kaplan-Meier method and the Cox proportional hazards model, assume that competing events are absent, and that censored participants would eventually have the outcome of interest if the study had continued for long enough. Using these approaches to analyse time-to-event data and censoring participants who have a competing event violates this assumption. This results in an overestimation of the probability of the outcome and misestimation of the magnitude of relative effects of predictors on the incidence of the event.[349-351] Many studies with competing events erroneously use these traditional approaches to time-to-event analysis.[358] Instead, I used competing risks time-to-event analyses to take into account the substantial competing event of death. I used the cumulative incidence function to estimate the incidence of recurrent ICH while accounting for death as a competing event.[349] I used two methods to calculate the hazard function. The subdistribution hazard function[353] represents the instantaneous rate of occurrence of the given event type in participants who have not yet experienced that event type. It estimates the effect of variables on the probability of events occurring over time and is recommended when assessing the impact of variables on the incidence of the outcome of interest or assessing prognosis.[351] The cause-specific hazard function[351, 352] represents the instantaneous rate of occurrence of an event among participants who are still event free. It is better for the assessment of aetiology of a disease process.[351] Reporting all three measures gives a better understanding of the effects of variables on the event of interest and on any competing event.[359]

I pre-specified the primary and secondary analyses and restricted the number of variables in the models to ensure at least 7-10 outcomes per

variable, to help reduce over-fitting. I ensured variables obeyed the proportional hazard assumptions before including them in multivariable analyses. I restricted the primary analyses to first-ever lobar ICH, to standardise the inception point. I reported the cumulative incidence function as well as subdistribution and cause-specific hazard models for recurrent ICH and death.[359]

10.5.3 Limitations of the studies

Despite using three ICH cohorts, the sample sizes were modest. In LATCH, I included 120 participants with first-ever lobar ICH, 17 of whom had a recurrent ICH during follow up. In CROMIS-2, 342 participants had a first-ever lobar ICH, 25 of whom had a recurrent ICH. The number of participants in the Edinburgh CT-APOE study with recurrent ICH was smaller (14 in LINCHPIN, 24 in CROMIS-2-DNA). The modest number of outcome events limited the number of variables I could include in the primary multivariable models. Therefore, I was unable to adjust for potentially important variables, such as age, and individual CT biomarkers of SVDs.

I was unable to adjust for key confounders of recurrent ICH, such as blood pressure control and antithrombotic use because accurate data on these variables during follow-up was not available in the cohorts. Therefore, I cannot exclude the influence of residual confounding on my results. Descriptive analyses showed the use of antihypertensive and antithrombotic drug at hospital discharge was similar between the Edinburgh CT-only and CT-APOE risk categories in the LATCH, LINCHPIN and CROMIS-2-DNA cohorts. However, the use of antihypertensive drugs at hospital discharge was lower in the high-risk CT-only group compared to the low risk in the CROMIS-2 cohort.

The non-contrast CT brain scans from the cohorts were assessed independently. There was no assessment of inter-rater agreement. Therefore, the differences in the magnitude of CT based predictors, such as CT SVD score and Edinburgh CT criteria found in the different cohorts may relate to differences in the rating of these features.

10.5.4 Study findings

10.5.4.1 Lobar ICH location is associated with a higher risk of recurrent ICH

In LATCH and CROMIS-2 participants with a first-ever SVD-associated ICH, a lobar index ICH location was associated with a significantly increased rate and incidence of recurrent ICH compared with a non-lobar index ICH location (Table 10.3, Figure 10.4 and Figure 10.5). The rate and incidence of death were similar between lobar and non-lobar ICH locations. Therefore, the effect of lobar ICH location on recurrent ICH is likely to represent a direct effect, rather than an indirect effect on the competing event of death. This finding is consistent with previous studies.[151] It is thought to reflect the likely underlying SVDs, with CAA, which is associated with approximately 60% of lobar ICH, understood to have a higher risk of recurrent ICH than arteriolosclerosis, which is associated with all non-lobar ICH, as well as a proportion of lobar ICH (Figure 4.13).[105, 154-157] To investigate this further, I assessed the association between the Edinburgh diagnostic criteria for CAA-associated lobar ICH and recurrent ICH.

10.5.4.2 Edinburgh CT-only diagnostic criteria for CAA-associated lobar ICH and the risk of recurrent ICH

In multivariable analyses of LATCH patients with first-ever lobar ICH, the Edinburgh CT-only CAA criteria high-risk group was associated with a significantly increased rate and incidence of recurrent ICH compared with the low-risk group (Table 10.8). There was no significant increase in the rate or incidence of death in the high-risk group, indicating that the effect on recurrent ICH is a direct effect, rather than an indirect effect on the competing event of death. The Edinburgh CT-only high-risk group showed a similar direction of effect in the multivariable models using CROMIS-2 participants with first-ever lobar ICH, although the associations were not statistically significant. In CROMIS-2, a CT SVD score of 1, 2 or 3 was associated with a significantly increased rate and incidence of both recurrent ICH and death (Table 10.9). It is difficult to determine if the effect on recurrent ICH is a direct or indirect effect through the competing event, given the similar magnitude of

effect on death. Two-step meta-analysis of the LATCH and CROMIS-2 cohorts showed a significantly increased incidence of recurrent ICH in the high-risk Edinburgh CT-only group relative to the low-risk group (subdistribution hazard ratio 2.99, 95%CI 1.08-8.27, Figure 10.8). These results suggest that CT markers of CAA-associated lobar ICH may be able to identify lobar ICH patients at high risk of recurrent ICH.

A recent meta-analysis assessed the risk of recurrent ICH in 1306 survivors of ICH, stratified by the likely underlying SVDs using MRI.[105] They compared participants with strictly lobar ICHs and CMBs (probable CAA and possible CAA on the original Boston criteria[103] – “CAA-related ICH”) against those with strictly deep or mixed ICHs and CMBs (“CAA-unrelated ICH”). Participants with CAA-related ICH had a seven-fold increase in the risk of recurrent ICH compared with CAA-unrelated ICH. In the CAA-related ICH group, the presence of lobar CMBs was associated with an increased risk of recurrent ICH. However, there were several limitations to this study, including selection bias, variable follow-up methods, few outcome events and possible confounding. The authors did not restrict their analyses to lobar ICH, nor to those with first-ever ICH. Also, no account was taken of the competing risk of death. Therefore, the probability of the outcome may have been overestimated. Nonetheless, this data support my findings that imaging biomarkers of CAA-associated lobar ICH may be helpful for identifying ICH patients at higher risk of recurrent ICH.

In a pre-specified secondary analysis, I assessed the risk of recurrent ICH stratified by the individual components of the Edinburgh CT-only criteria. Subarachnoid haemorrhage significantly increased the rate and incidence of recurrent ICH, adjusting for age, sex, previous history of ICH, antihypertensive drug use on hospital discharge and CT SVD score (Table 10.13). The presence of finger-like projections was associated with a non-significant increase in the rate and incidence of recurrent ICH.

Cortical superficial siderosis is an MRI biomarker of CAA.[107, 110] A recent meta-analysis of 443 CAA-associated lobar ICH survivors showed that

cortical superficial siderosis is an independent predictor for recurrent ICH.[116] This association persisted after adjusting for age, sex, previous lobar ICH history, WMH volume and CMBs. Cortical superficial siderosis may, therefore, be a strong predictor of recurrent lobar ICH. However, this study was small and prone to selection bias and confounding. The authors postulate that cortical superficial siderosis is a marker of increased cortical and leptomeningeal small vessel fragility, resulting in a high risk of recurrent ICH.[116, 360, 361] Subarachnoid haemorrhage is the putative acute manifestation of cortical superficial siderosis.[362] Therefore, the association in my study between subarachnoid haemorrhage and the risk of recurrent ICH supports the premise of CAA affecting the superficial cortical and leptomeningeal small vessels results in a high risk of recurrent ICH.

10.5.4.3 Edinburgh CT-APOE diagnostic criteria for CAA-associated lobar ICH and the risk of recurrent ICH

The Edinburgh CT-APOE diagnostic criteria variable did not obey the proportional hazards assumption in the LINCHPIN cohort, and there was insufficient power to allow me to include covariate time interaction terms. Therefore, I did not perform the primary two-step meta-analysis. In a pre-specified one-step meta-analysis of the LINCHPIN and CROMIS-2-DNA cohorts, the high-risk group on the Edinburgh CT-APOE CAA criteria was significantly associated with an increased rate and incidence of recurrent ICH compared with the low-risk group (Table 10.19). There was no significant increase in the rate or incidence of death in the high-risk group, indicating that the effect on recurrent ICH is a direct effect, rather than an indirect effect of the competing event of death. A CT SVD score of 1, 2 or 3 was significantly associated with an increased rate and incidence of both recurrent ICH and death (Table 10.19). It is difficult to determine if the effect on recurrent ICH is a direct or indirect effect through the competing event, given the similar magnitude of effect on death.

In a pre-specified secondary analysis, APOE $\varepsilon 2$ allele possession was significantly associated with an increased rate and incidence of recurrent

ICH, while the high-risk Edinburgh CT-APOE category had a borderline significant association with the risk of recurrent ICH (Table 10.21).

It has been proposed that APOE ε4 is associated with increased vascular deposition of amyloid-β and APOE ε2 promotes vasculopathic wall changes.[363-365] There is some evidence that APOE genotype is associated with the severity of CAA. A systematic review of pathologically proven CAA revealed a possible association of severe CAA with APOE ε4.[25] APOE ε2 was associated with more severe CAA. However this did not reach significance, which may reflect the small numbers of participants in this part of the study.[25] The possession of an APOE ε2 or ε4 allele has been shown to be associated with increased risk of recurrent ICH,[366, 367] although these studies were prone to confounding and did not account for death as a competing risk. In line with these studies, my results suggest that APOE ε2 allele possession is associated with an increased risk of recurrent ICH, while there may be a possible association between APOE ε4 allele possession and recurrent ICH risk. However, the sample size in my study was limited.

10.5.5 Clinical implications

My results suggest that simple features on the diagnostic non-contrast brain CT (the Edinburgh CT-only criteria and CT SVD score) may be able to identify ICH patients at high and low risk of recurrent ICH. APOE genotype may also be associated with an increased risk of recurrent ICH. This information could help with prognosis and might help guide treatment decisions,[368-370] but only after the findings are replicated in larger cohorts of ICH.

MRI biomarkers of CAA are potentially useful for identifying ICH patients with a higher risk of recurrent ICH.[105, 116] However, many ICH patients may not have access to or be able to tolerate MRI scanning. Therefore, predicting recurrent ICH risk using simple tests is important. Non-contrast brain CT is usually the first test to diagnose ICH and is the most widely available neuroimaging test. APOE genotype can be performed on a peripheral blood

sample. My studies indicate that non-contrast brain CT with or without APOE genotyping may be useful and easily available tests for identifying ICH patients at higher risk of recurrent ICH.

10.5.6 Future directions

These results need to be assessed in larger studies of ICH survivors with comprehensive follow-up for recurrent ICH and death. The time-to-event analyses should take into account the substantial competing risk of death. The analyses should be adjusted for important confounders, such as age, history of previous ICH and antithrombotic drug use and blood pressure control during follow-up.

I am currently coordinating a multicentre study assessing the association between the Edinburgh CT-only and CT-APOE diagnostic criteria and the risk of recurrent ICH. There are eight European and North American contributing centres involved, with over 2000 participants with first-ever lobar ICH, diagnostic non-contrast brain CT and follow-up for recurrent ICH and death. Around 1700 participants also have APOE genotyping. This large multicentre study should provide sufficient power to assess the prognostic value of the Edinburgh diagnostic criteria while adjusting for important confounders.

The collaborators in this multicentre study are assessing the diagnostic brain CTs in their own cohorts for the Edinburgh criteria. Before rating the imaging, they are undertaking the online training for the Edinburgh criteria which I have developed.[330] This will hopefully improve the reliability of the ratings. Also, there are 60 practice cases within the training materials, which will allow me to assess inter-rater agreement between the collaborators, as well as any other researchers who have undertaken the training.

ICH survivors are at risk of future ischaemic events, as well as recurrent ICH,[151] and may therefore benefit from antithrombotic drugs. However, the risk of antithrombotic-associated recurrent ICH in survivors of CAA-associated lobar ICH is uncertain. Biffi et al. assessed the associations with risk of recurrent ICH in CAA-associated lobar ICH.[158] They showed that a previous lobar ICH, two or more lobar CMBs, posterior white matter lucencies

on CT and aspirin use after the index ICH were independently associated with the risk of recurrent ICH. However, this was a single centre cohort study. Only those who underwent a MRI were included, which is likely to introduce a selection bias. The sample size was modest, with only 29 recurrent ICHs during follow-up, and the regression models were over-fitted. The authors did not account for death as a competing risk, which will overestimate the strength of the reported associations. Finally, the results may be the result of confounding as this was not a randomised study. In particular, the authors did not account for blood pressure control during follow-up, which is known to affect the risk of recurrent ICH.[159] In contrast, a recent randomised controlled trial assessing the risk of recurrent ICH between SVD-associated ICH survivors showed that the group restarted on aspirin had a borderline significantly lower risk of recurrent ICH compared with the group not restarted on aspirin.[160] Furthermore, there was also no statistically significant association between CT or MRI markers of CAA and treatment group effect, suggesting that the finding of imaging biomarkers of CAA does not increase the risk of antithrombotic-associated ICH. However, the power of these subgroup analyses was limited, and there is likely to be selection bias in the study.[161] It is therefore unclear whether aspirin increases the risk of recurrent ICH in survivors of lobar ICH when imaging biomarkers of CAA are present. Sufficiently powered randomised controlled studies should aim to address whether imaging biomarkers of CAA are associated with increased risk of recurrent antithrombotic-associated ICH.

Chapter 11 Prediction models for death and disability at one year after first-ever SVD-associated ICH

11.1 Introduction

Accurately predicting outcomes in SVD-associated ICH is important. It can help improve communication between clinicians, be used by clinicians to discuss prognosis with patients and their relatives and may help guide treatment decisions. Such information can also be used to select patients for clinical trials.[371-373] Prediction models may help improve prognostication beyond informal clinical assessment through rigorous development and external validation. Many prognostic tools have been developed for ICH,[373-376] of which the ICH score is the most commonly used.[244]

The ICH score is a simple ordinal grading scale based on five categorical predictors (age [<80 versus ≥80 years], admission GCS [3-4, 5-12 or 13-15], infratentorial ICH location, ICH volume [<30 ml versus ≥30 ml] and presence of intraventricular haemorrhage). It was derived from a logistic regression model for 30-day fatality developed in a retrospective single centre cohort consisting of 152 participants with ICH.[244] It has been externally validated for short (≤1 month)[245, 377-384] and long term (≥1 month)[380, 385] fatality, as well as short (≤1 month)[382, 386] and long term functional outcome(≥1 month)[380, 385-390] in a variety of healthcare settings.

Whilst the ICH score has many strengths, there are some limitations. Firstly, it was developed on a relatively small Californian cohort from the 1990s. The magnitude of the included predictor associations may be different in ICH patients from the UK, and they may have changed in the intervening 20 years. Also, new predictors have been identified that are associated with outcome after ICH,[151] such as white matter lucencies and other CT features of SVD.[195, 391] However, the prognostic value of CT SVD biomarkers in ICH is not fully understood. Some of the approaches the

authors took to model development, such as only including significant variables and converting continuous variables into categorical predictors, will have had a detrimental effect on predictive power.[334] Finally, the ICH score was specifically developed to predict short term fatality after ICH. 30-day fatality is high after ICH, around 40%,[133] and therefore a useful outcome to predict. However, fatality after ICH continues to increase beyond the short term, with population studies showing that it reaches 54% by one year.[151] The ICH-score shows good discrimination for 30-day mortality (pooled c statistic of the ICH score is 0.81, 95%CI 0.76-0.86)[375] and moderate discrimination for 90-120-day mortality (pooled c statistic 0.79, 95%CI 0.70-0.88).[375] However, functional outcome is probably a more relevant clinical outcome than fatality; patients, relatives and medical practitioners are usually more interested in the chances of functional recovery rather than just survival after ICH.[371, 392]

Several prediction models, such as the FUNC score,[152] modified ICH score[393] and Essen ICH score,[394] have been developed to specifically assess long term functional outcome (≥1 month). These scores are based on similar predictors to the ICH score, such as age, neurological status, ICH location and ICH volume, with reclassification of some of these and/or additional predictors included. They all have methodological limitations, such as selection bias and relatively high levels of missing outcome data. Also, they were all derived on cohorts from the 1990s or early 2000s. Finally, they were developed to assess functional outcome between 90-180 days, however functional recovery continues to evolve beyond the acute and intermediate phases, with changes occurring throughout the first year after ICH.[387] The ICH Functional Outcome Score (ICH-FOS) was developed to predict one-year functional outcome after ICH and performed well against existing scores.[390] However, this study excluded relevant ICH participants, such as those with pre-ICH disabilities. It was developed in a large cohort in China, and the demographics and some of the ICH characteristics of those included differ to patients in LATCH. Therefore, its relevance to patients with ICH in the UK is unclear.

Developing a prognostic prediction model for ICH in a large, unselected contemporary ICH cohort using a rigorous methodological approach to prediction modelling is important. In addition to the established clinical and radiological predictors of survival, CT biomarkers of SVD severity may add prognostic value and their incremental value should be assessed. The most clinically relevant model outcomes are fatality and functional recovery beyond the short term. Comparison of the performance of any novel prediction model against the ICH score is important since the ICH score is the most extensively validated prognostic score in ICH and there is a lack of evidence of superior predictions by other prognostic models.[395]

The LATCH cohort is a large, representative ICH cohort with low levels of missing baseline data and comprehensive follow-up for death and functional status. It, therefore, provides an excellent dataset for developing a clinically relevant prediction model for death and disability after SVD-associated ICH.

11.2 Aims

I aimed to:

- Assess survival in first-ever SVD-associated ICH.
- Develop multivariable logistic regression prognostic models to predict the risk of death and death or disability at one year after first-ever SVD-associated ICH and to compare these against logistic regression models based on the ICH score.[244]
- Perform a pragmatic update to the ICH score by incorporating the CT SVD score, and to compare its performance against the original ICH score for predicting death and death or disability at one year after first-ever SVD-associated ICH.

11.3 Methods

I performed and reported the study according to the TRIPOD guidelines.[332]

11.3.1 Study design and patients

I used data from the prospective, community-based LATCH study (section 2.1.3.1). I included consecutive adult patients (aged ≥16 years) living in the NHS Lothian Health Board region who had a first-ever spontaneous ICH between 1st June 2010 and 31st May 2013 inclusive.

I excluded those with exclusively extra-axial intracranial haemorrhage and ICH secondary to an underlying cause other than SVDs. I also excluded patients with a previous symptomatic ICH.

11.3.1.1 Treatments

Patients received standard clinical care, including admission to a dedicated stroke unit where possible, rehabilitation and secondary prevention through treatment of hypertension with antihypertensive drugs.[177, 178] Decisions regarding do not attempt resuscitation orders and withdrawal of active care were made by the clinical team and the patient and their relatives based on clinical information and patient/relative wishes; there were no local guidelines for these interventions.

11.3.2 Predictors

The RUSH team collected demographics and the presence of relevant co-morbidities and medication use at the time of ICH as described in section 2.1.7.

I assessed the diagnostic non-contrast brain CT using a standardised pro forma, as described in section 2.2.1.3. I evaluated the presence, number and location of acute ICHs,[190] the volume of each ICH using a modified ABC/2 approach,[191] and the presence or absence of extra-axial haemorrhage (subarachnoid, subdural or intraventricular spaces) and finger-like projections arising from the largest haematoma. I calculated the CT SVD burden score[196] based on the presence and severity of white matter lucencies[193] and cerebral atrophy[195], the number of lacunes[77] and CT SVD burden score[196] as described in the methods (2.2.1.3).

I performed the CT ratings masked to clinical, MRI and outcome information.

11.3.3 Outcome

The RUSH team used multiple sources of information to follow-up patients in LATCH as described in section 2.1.8. For this study, I censored follow-up data at five years after the index ICH or on 27th February 2018, whichever occurred first.

I pre-specified the outcomes of interest in this study:

1. Death from any cause at one year after the index ICH
2. Death from any cause or disability, defined as a modified Rankin Scale 4-6,[185] at one year after the index ICH.

I chose the outcome of death from any cause at one year after the index ICH because ICH is associated with high fatality.[133] Case fatality is highest in the early post-ICH period but continues to increase during the first year after ICH.[151] Therefore one-year fatality is likely to be a more useful outcome than 30-day fatality.

I chose the combined outcome of death or disability at one year after the index ICH because the functional outcome is probably a more relevant clinical outcome than fatality alone.[371, 392] I chose the one year time point because functional outcome continues to evolve throughout the first year after ICH.[387] I dichotomised this outcome (modified Rankin Scale 0-3 versus 4-6) to increase the power for analysis and to allow me to compare my results with other studies.[386-388]

Deaths were adjudicated by a consultant neurologist with an interest in stroke medicine (Prof R Al-Shahi Salman) using the death certificate and GP and hospital records to confirm the cause and date of death. Adjudication was performed masked to the CT ratings, including the CT SVD score.

The one year modified Rankin Scale was ascertained in surviving patients through a postal questionnaire sent to the patients' GPs. The GPs used all available clinical information to derive the modified Rankin Scale. The GPs were not aware of this study when assessing the modified Rankin Scale.

11.3.4 Sample size

I did not perform a sample size calculation because there is no generally accepted approach to estimating sample size in prediction modelling.[333] Instead, I used the largest sample size possible from the three-year community-based LATCH study to maximise power and generalisability. I reduced overfitting by following the general guidance of pre-specifying predictors, having at least 10 outcome events per predictor and at least 100 events.[333]

11.3.5 Missing data

I excluded 15 patients with first-ever SVD-associated ICH because they did not have a diagnostic non-contrast CT brain scan. Information about pre-ICH history of diabetes was missing for one patient, and the admission GCS was not available for two patients. The modified Rankin scale at one year after the index ICH was missing in 10 patients. I excluded patients with missing predictors or outcomes from the relevant analyses. I did not impute any missing data.

11.3.6 Statistical analysis

11.3.6.1 Survival analysis

I quantified the follow-up time using the median and interquartile range, and the completeness of follow-up by calculating the completeness index ([observed follow up/potential follow up]*100).[348]

I performed univariable Cox regression to assess for associations between survival time and pre-specified variables (age, sex, pre-ICH diagnosis of dementia, pre-ICH diagnosis of diabetes, antiplatelet use at the time of the ICH, anticoagulant use at the time of the ICH, admission GCS, ICH location (lobar versus non-lobar), ICH volume, intraventricular haemorrhage and CT SVD score ≥1) associated with long-term survival and functional outcome in ICH.[151, 152, 195, 391] I used Kaplan-Meier plots to show the survival curves for categorical predictors. I dichotomized the CT SVD score 0 versus ≥1 for analyses for two reasons. First, I wanted to differentiate those without evidence of severe SVD on CT (CT SVD score 0) from those with severe CT

SVD (≥1). Secondly, I wanted to maximise power in my analyses, and very few participants had CT SVD score of 3.

11.3.6.2 Prediction modelling and performance

For the two outcomes, death and death or disability at one year after ICH, I compared the frequency of clinical characteristics and diagnostic non-contrast brain CT features between groups using χ^2 test (or Fisher's exact test, where appropriate) for categorical variables and the Mann-Whitney U test for non-normally distributed continuous variables.

I developed three separate prediction models for each outcome using logistic regression. For the first model ("full pre-specified model") I included 11 pre-specified predictors. Ten of these (age, sex, pre-ICH diagnosis of dementia, pre-ICH diagnosis of diabetes, antiplatelet use at the time of the ICH, anticoagulant use at the time of the ICH, admission GCS, ICH location (lobar versus non-lobar), ICH volume and intraventricular haemorrhage) were based on the variables most frequently associated with long-term survival and functional outcome in ICH.[151, 152] I also included the predictor CT SVD score ≥1 based on recent evidence of an association between CT SVD biomarkers and outcome in ICH.[195, 391] I modelled the continuous predictors age, GCS on admission and ICH volume as linear associations as there was no evidence of nonlinearity. There was no evidence of interactions between ICH location, ICH volume and CT SVD score.

For the second model ("ICH-SVD score model") I included six pre-specified predictors. Five were based on the variables included in the logistic regression model used to derive the ICH score (age, admission GCS, infratentorial ICH location, ICH volume and presence of intraventricular haemorrhage).[244] The other variable was CT SVD score (0 or ≥1). I dichotomised age (<80 or ≥80 years), but kept GCS and ICH volume as continuous linear variables, as this was the approach taken by Hemphill et al. in their logistic regression model.[244]

I wanted to compare my new prediction models against the ICH score model. However, the logistic regression equation for the ICH score was not included in the published paper[244] nor was it available after contacting the

corresponding author. Therefore, I fitted logistic regression models for death and death or disability at one year in the LATCH data using the ICH score variables used in the original ICH score logistic regression model, as described in the above paragraph (“ICH score model”).

Predictions from multivariable models can be improved for new subjects by shrinkage of the regression coefficients to reduce variance.[334, 396, 397] I used penalised maximum likelihood estimation, which uses a penalty factor in the estimation of coefficients, to shrink the logistic regression coefficients of the prediction models. The optimal penalty factor was determined by the Akaike information criterion (AIC).[398]

For each shrunken model, I evaluated its overall performance using Nagelkerke’s R^2, the Brier score and AIC.[339] The AIC assesses model fit while taking into account the number of predictors in the model. The lower the AIC, the better the model fit. It can be used to compare the fit of different logistic regression models. I assessed model discrimination with the c statistic and displayed this graphically using ROC plots. I assessed model calibration using calibration plots.

I internally validated each shrunken prediction model with bootstrapping by drawing 2000 random bootstrap samples with replacement, constructed models on each of these bootstrap samples and derived optimism-adjusted measures of performance.[339, 399]

11.3.6.3 ICH score and ICH-SVD score

I developed a modified ICH score (ICH-SVD score) by adding the predictor CT SVD score to the ICH score. I assigned one point to a CT SVD score ≥1 based on the magnitude of its coefficient relative to the other variables in the ICH-SVD score logistic regression model. My ICH-SVD score therefore ranged from 0 to a maximum of 7.

I compared the ICH-SVD score against the original ICH score for the two outcomes (death at one year and death or disability at one year) using the c statistic and displayed this graphically using ROC plots.

11.4 Results

11.4.1 Flow of patients

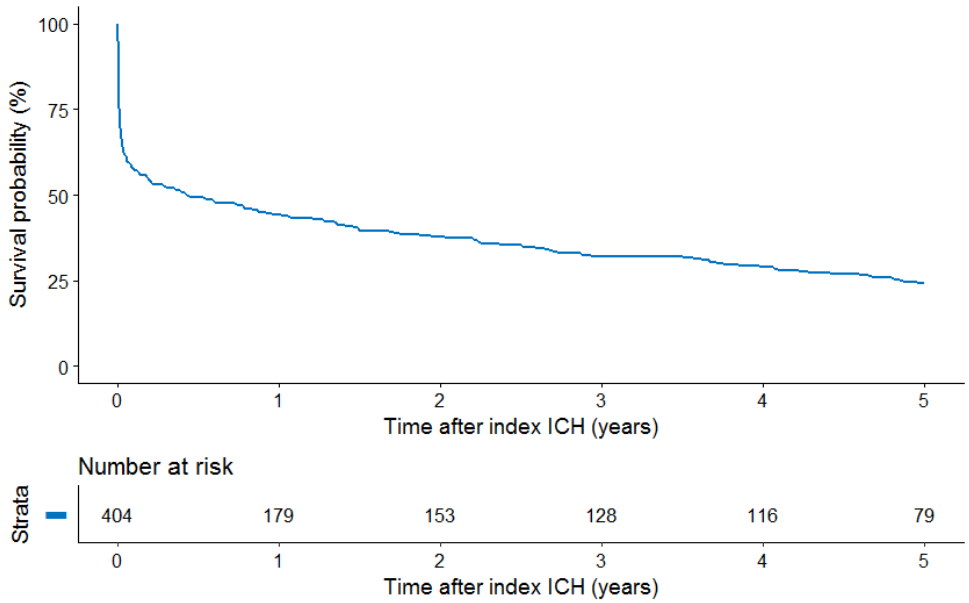
There were 530 patients with a spontaneous ICH in the NHS Lothian Health Board region between 1st June 2010 and 31st May 2013 inclusive. Four hundred nineteen had a first-ever SVD-associated ICH. I included 404 who had a diagnostic non-contrast brain CT (Figure 3.1).

11.4.2 Survival analysis

Three hundred and four of the 404 (75.2%) patients died during follow-up. The median duration of follow-up was 156 days (IQR 4-1547), with 98.1% completeness of follow-up.

By 30 days, 168 patients (42%, 95%CI 37-46%) had died (Figure 11.1). This increased to 225 patients (56%, 95%CI 51-60%) at one year and 304 patients (75%, 95%CI 71-80%) at five years. The Kaplan-Meier survival curves for the pre-specified categorical variables are shown in Figure 11.2.

Figure 11.1 Kaplan-Meier survival curve for first-ever SVD-associated ICH



ICH = intracerebral haemorrhage. SVD = small vessel disease.

Of the 11 pre-specified predictors, all except sex, anticoagulant use at the time of the index ICH and ICH location were associated with death on univariable Cox regression (Table 11.1).

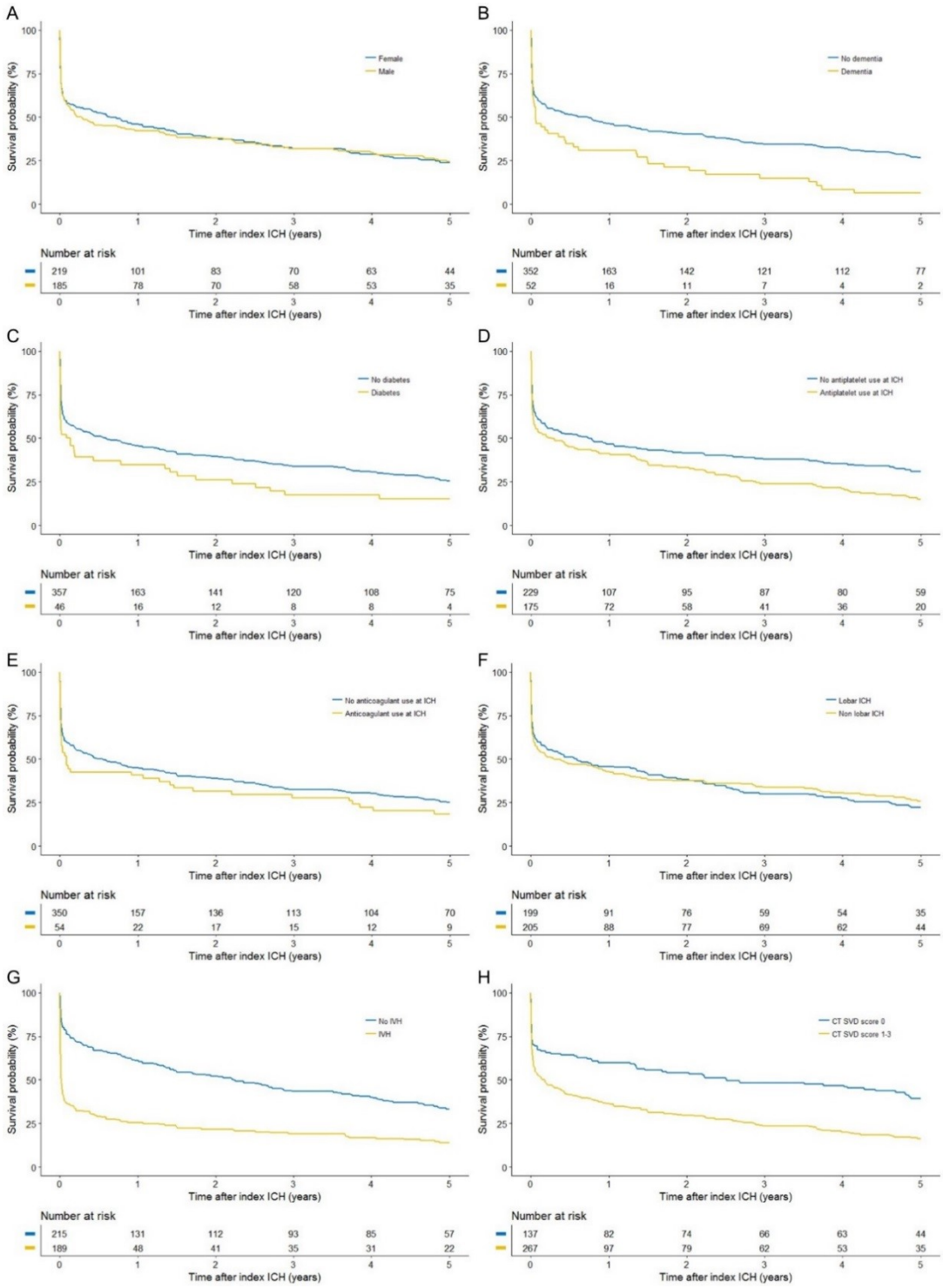
11.4.3 Prognostic models for death at one year after first-ever SVD-associated ICH

11.4.3.1 Baseline clinical and diagnostic CT brain characteristics

Two hundred and twenty-five patients (56%) were dead at one year after their index ICH whilst 179 patients were alive. Those who died were older, with a more frequent history of pre-ICH ischaemic stroke or dementia, had higher pre-ICH modified Rankin scale scores and lower GCS on admission (Table 11.2). Larger ICH volume and more frequent intraventricular haemorrhage, subarachnoid haemorrhage, subdural haemorrhage and finger-like projections were also associated with death at one year, as was more severe CT SVD score (Table 11.3). There was no univariable association between sex, pre-ICH diabetes, antiplatelet or anticoagulant drug use at the time of ICH and ICH location and death at one year.

Figure 11.2 Kaplan-Meier survival curves for pre-specified categorical variables in first-ever SVD-associated ICH.

A. Sex, B. Pre-ICH dementia, C. Pre-ICH diabetes, D. Antiplatelet use at the time of ICH, E. Anticoagulant use at the time of ICH, F. ICH location, G. intraventricular haemorrhage and H. CT SVD score.



CT = computed tomography. ICH = intracerebral haemorrhage. IVH = intraventricular haemorrhage. SVD = small vessel disease.

Table 11.1 Univariable cox regression for death in first-ever SVD-associated ICH

	Death during follow-up	**Hazard ratio**	**95%CI**	**p value**
Age (per year increase)		1.03	1.02-1.04	<0.001
Sex				
Female	166/219	Reference	-	-
Male	138/185	1.00	0.80-1.26	0.980
Pre-ICH dementia				
No	256/352	Reference	-	-
Yes	48/52	1.66	1.22-2.27	0.001
Pre-ICH diabetes				
No	264/357	Reference	-	-
Yes	39/46	1.51	1.08-2.11	0.019
Antiplatelet use at ICH				
No	157/229	Reference	-	-
Yes	147/175	1.42	1.14-1.78	0.002
Anticoagulant use at ICH				
No	260/350	Reference	-	-
Yes	44/54	1.24	0.90-1.71	0.186
Glasgow coma scale (per unit change)		0.82	0.79-0.84	<0.001
Non-lobar ICH location				
No	153/199	Reference	-	-
Yes	151/205	0.98	0.78-1.23	0.832
ICH volume (per ml)		1.01	1.01-1.02	<0.001
Intraventricular haemorrhage				
No	142/215	Reference	-	-
Yes	162/189	2.28	1.82-2.87	<0.001
CT SVD score				
0	82/137	Reference	-	-
≥1	222/267	1.89	1.46-2.43	<0.001

CT = computed tomography. ICH = intracerebral haemorrhage. SVD = small vessel disease.

Table 11.2 Baseline clinical features in first-ever SVD-associated ICH patients who were alive at one year after the index ICH versus those who were dead

	Alive at 1 year (n=179)		Dead at 1 year (n=225)		p value
Age (years); median (IQR)	74	(61-82)	79	(72-84)	<0.001
Sex					
Female	101	(56)	118	(52)	0.425
Male	78	(44)	107	(48)	
Co-morbidities					
Hypertension	113	(63)	153	(68)	0.276
Ischaemic stroke†	20	(11)	48	(21)	0.006
Transient ischaemic attack†	16	(9)	29	(13)	0.204
Dementia	16	(9)	36	(16)	0.035
Diabetes†	16	(9)	30	(13)	0.162
Atrial fibrillation†	39	(22)	50	(22)	0.898
Myocardial infarction	11	(6)	26	(12)	0.059
Hyperlipidaemia	28	(16)	43	(19)	0.352
Smoking status‡					
Current	36	(22)	50	(24)	
Ex-smoker	50	(31)	82	(39)	0.112
Never	76	(47)	76	(37)	
Pre-ICH modified Rankin scale					
0	81	(45)	48	(22)	
1	37	(21)	46	(21)	
2	30	(26)	58	(26)	
3	26	(25)	55	(25)	
4	5	(5)	10	(5)	
5	0	(0)	3	(1)	
Pre-ICH modified Rankin scale; median (IQR)	2	(1-3)	3	(2-4)	<0.001
Medications on admission					
Antiplatelet drug(s)	72	(40)	103	(46)	0.263
Anticoagulant drug(s)	22	(12)	32	(14)	0.571
Antihypertensive drug(s)	87	(49)	111	(49)	0.884
Admission GCS score; median (IQR)*	14	(14-15)	11	(6-14)	<0.001

Data are n (%), median (IQR) or mean (SD). † data missing in 1 patient . * data missing in 2 patients. ‡ data missing in 34. GCS = Glasgow coma scale. ICH = intracerebral haemorrhage. SVD = small vessel disease.

Table 11.3 Non-contrast brain CT features in first-ever SVD-associated ICH patients who were alive at one year after the index ICH versus those who were dead

	Alive at 1 year (n=179)		Dead at 1 year (n=225)		p value
ICH location					
Lobar	91	(51)	108	(48)	
Deep	69	(39)	85	(38)	0.547
Infratentorial	19	(11)	32	(14)	
ICH volume (ml); median (IQR)	10	(3-22)	38	(13-83)	<0.001
Intraventricular haemorrhage	48	(27)	141	(63)	<0.001
Subarachnoid haemorrhage	65	(36)	111	(49)	0.009
Subdural haemorrhage	12	(7)	30	(13)	0.030
Finger-like projections	11	(6)	33	(15)	0.006
Number of lacunes; median (IQR)	0	(0-1)	0	(0-1)	0.653
Anterior white matter lucencies					
0	42	(24)	37	(16)	
1	100	(56)	104	(46)	0.001
2	37	(21)	84	(37)	
Posterior white matter lucencies					
0	71	(40)	53	(24)	
1	41	(23)	53	(24)	0.001
2	67	(37)	119	(53)	
Central atrophy					
0	56	(31)	46	(20)	
1	110	(62)	131	(58)	<0.001
2	13	(7)	48	(21)	
Cortical atrophy					
0	51	(29)	55	(24)	
1	100	(56)	110	(49)	0.028
2	28	(16)	60	(27)	
CT SVD score					
0	82	(46)	55	(24)	
1	61	(34)	94	(42)	<0.001
2	29	(16)	62	(28)	
3	7	(4)	14	(6)	

Data are n (%), median (IQR) or mean (SD). CT = computed tomography. ICH = intracerebral haemorrhage. SVD = small vessel disease.

11.4.3.2 Multivariable logistic regression prediction models

I excluded one patient because of missing data for pre-ICH diabetes and two for missing admission GCS score. Therefore, I included 401 patients who had complete data for all predictors and the outcome.

Full pre-specified model

The full prediction model for death included 11 pre-specified predictors and is shown in Table 11.4. The variance inflation factor values ranged from 1.06 to 1.50, confirming no evidence of multicollinearity between the predictors.

The shrunken model calculates the predicted probability of death at one year after index ICH as follows:

$$\begin{aligned} Risk\ score = & -2.41 + 0.04 * (age) + 0.59 * (male\ sex) - 0.06 \\ & * (pre - ICH\ dementia) + 0.31 * (pre - ICH\ diabetes) - 0.32 \\ & * (antiplatelet\ use) - 0.01 * (anticoagulant\ use) - 0.20 \\ & * (GCS) + 0.50 * (non\ lobar\ ICH\ location) + 0.02 \\ & * (ICH\ volume\ ) + 0.63 * (intraventricular\ haemorrhage) \\ & + 0.76 * (CT\ SVD\ score \geq 1) \end{aligned}$$

$$Predicted\ probability = \frac{1}{1 + exp^{-risk\ score}}$$

The categorical predictor values are 1 when present and 0 when absent, age is the age at the time of the index ICH in years, GCS is the GCS score on hospital admission and ICH volume is the volume of the largest ICH in ml.

ICH score model

The logistic regression prediction model for the variables used in the ICH score logistic regression model is shown in Table 11.5. The variance inflation factor values ranged from 1.01 to 1.13, confirming no evidence of multicollinearity between the predictors.

The shrunken model calculates the predicted probability of death at one year after index ICH as follows:

$$Risk\ score = 1.45 + 0.64 * (age) - 0.20 * (GCS) + 0.10 * (infratentorial\ ICH\ location) + 0.02 * (ICH\ volume) + 0.76 * (intraventricular\ haemorrhage)$$

$$Predicted\ probability = \frac{1}{1 + exp^{-risk\ score}}$$

The value of the predictor age is 1 when the age is ≥80 years and 0 when age is <80 years. The other categorical predictor values are 1 when present and 0 when absent. ICH volume is the volume of the largest ICH in ml and GCS is GCS score on hospital admission.

Table 11.4 Full pre-specified multivariable logistic regression prediction models for death at one year after the index ICH in first-ever SVD-associated ICH

	Original model			Shrunken model		
	β Coefficient (standard error)	Odds ratio (95%CI)	p value	β Coefficient (standard error)	Odds ratio (95%CI)	p value
Intercept	-3.05 (1.20)			-2.41 (1.08)		
Age (per year increase)	0.05 (0.01)	1.05 (1.02-1.08)	<0.001	0.04 (0.01)	1.04 (1.02-1.07)	<0.001
Male sex	0.72 (0.27)	2.05 (1.20-3.51)	0.008	0.59 (0.25)	1.81 (1.12-2.92)	0.016
Pre-ICH dementia	-0.14 (0.41)	0.87 (0.39-1.96)	0.736	-0.06 (0.35)	0.95 (0.48-1.87)	0.874
Pre-ICH diabetes	0.44 (0.52)	1.55 (0.69-3.51)	0.288	0.31 (0.35)	1.36 (0.69-2.69)	0.381
Antiplatelet use at ICH	-0.46 (0.30)	0.63 (0.35-1.14)	0.127	-0.32 (0.26)	0.83 (0.44-1.21)	0.223
Anticoagulant use at ICH	-0.07 (0.42)	0.93 (0.41-2.12)	0.863	-0.01 (0.36)	0.99 (0.50-1.94)	0.969
Glasgow coma scale (per point increase)	-0.21 (0.05)	0.81 (0.73-0.89)	<0.001	-0.20 (0.04)	0.82 (0.75-0.89)	<0.001
Non lobar ICH location	0.65 (0.30)	1.91 (1.07-3.41)	0.028	0.50 (0.26)	1.65 (0.99-2.74)	0.052
ICH volume (per ml increase)	0.03 (0.01)	1.03 (1.02-1.04)	<0.001	0.02 (0.00)	1.02 (1.01-1.03)	<0.001
Intraventricular haemorrhage	0.63 (0.29)	1.87 (1.06-3.29)	0.030	0.63 (0.26)	1.88 (1.14-3.10)	0.014
CT SVD score ≥1	0.91 (0.29)	2.48 (1.39-4.41)	0.002	0.76 (0.26)	2.15 (1.29-3.58)	0.003

CT = computed tomography. ICH = intracerebral haemorrhage. SVD = small vessel disease.

Table 11.5 ICH score multivariable logistic regression prediction models for death at one year after the index ICH in first-ever SVD-associated ICH

	Original model			Shrunken model		
	β Coefficient (standard error)	Odds ratio (95%CI)	p value	β Coefficient (standard error)	Odds ratio (95%CI)	p value
Intercept	1.56 (0.70)			1.45 (0.65)		
Age ≥80	0.67 (0.25)	1.96 (1.21-3.20)	0.007	0.64 (0.24)	1.89 (1.18-3.03)	0.008
Glasgow coma scale (per point increase)	-0.21 (0.05)	0.81 (0.74-0.89)	<0.001	-0.20 (0.04)	0.82 (0.75-0.89)	<0.001
Infratentorial ICH location	0.45 (0.38)	1.57 (0.75-3.27)	0.233	0.10 (0.36)	1.51 (0.74-3.08)	0.254
ICH volume (per ml increase)	0.02 (0.00)	1.02 (1.01-1.03)	<0.001	0.02 (0.00)	1.02 (1.01-1.02)	<0.001
Intraventricular haemorrhage	0.76 (0.26)	2.13 (1.28-3.54)	0.004	0.76 (0.25)	2.14 (1.31-3.49)	0.002

ICH = intracerebral haemorrhage. SVD = small vessel disease.

ICH-SVD score model

I combined the CT SVD score predictor with the other variables from the ICH score logistic regression model to create a modified ICH (ICH-SVD) score logistic regression prediction model (Table 11.6). The variance inflation factor values ranged from 1.04 to 1.19, indicating no evidence of multicollinearity between the predictors.

The shrunken model calculates the predicted probability of death at one year after index ICH as follows:

$$Risk\ score = 0.80 + 0.46 * (age) - 0.20 * (GCS) + 0.41 * (infratentorial\ ICH\ location) + 0.02 * (ICH\ volume) + 0.72 * (intraventricular\ haemorrhage) + 0.98 * (CT\ SVD\ score \geq 1)$$

$$Predicted\ probability = \frac{1}{1 + exp^{-risk\ score}}$$

The value of the predictor age is 1 when the age is ≥80 years and 0 when age is <80 years. The other categorical predictor values are 1 when present and 0 when absent. ICH volume is the volume of the largest ICH in ml and GCS is GCS score on hospital admission.

11.4.3.3 Performance of the shrunken prediction models

The performance measures of the three models are summarised in Table 11.7. The full pre-specified model showed good discrimination (c statistic 0.86, 95%CI 0.82-0.89) and good calibration (Figure 11.3). The ICH score model showed good discrimination (c statistic 0.82, 95%CI 0.78-0.86) and no evidence of miscalibration (Figure 11.4). The ICH-SVD score model also showed good discrimination (c statistic 0.84, 95%CI 0.80-0.88) and calibration (Figure 11.5). The AIC was best for the full pre-specified model (407.8), followed by the ICH-SVD model (413.5) then the ICH score model (427.6).

Internal validation resulted in small optimism-adjusted differences in the overall performance measures and discrimination for all three models (Table 11.7), with no evidence of poor calibration (Figure 11.3 to Figure 11.5).

11.4.4 ICH score versus the ICH-SVD score

The ICH score had a c statistic of 0.79 (95%CI 0.74-0.83). The addition of the predictor CT SVD ≥1 (ICH-SVD score) slightly increased the c statistic to 0.80 (95%CI 0.76-0.84) (Figure 11.6).

The percentage of patients who were dead at one year after the index ICH increased with both the ICH score and ICH-SVD score (Figure 11.7). Twenty-three percent (95%CI 15-33%) of patients with an ICH score of 0 were dead at one year. Ninety-three percent of patients (95%CI 82-98%) with an ICH score of 4 died within the first year, whereas 100% (95%CI 82-100%) with an ICH score of 5 or 6 had died.

Eleven percent (95%CI 4-25%) of patients with an ICH-SVD score of 0 were dead at one year. Ninety-five percent of patients (95%CI 82-99%) with an ICH-SVD score of 5 died within the first year, and 100% (95%CI 78-100%) with an ICH-SVD score of 6 or 7 had died.

Table 11.6 ICH-SVD score multivariable logistic regression prediction models for death at one year after the index ICH in first-ever SVD-associated ICH

	Original model			Shrunken model		
	β Coefficient (standard error)	Odds ratio (95%CI)	p value	β Coefficient (standard error)	Odds ratio (95%CI)	p value
Intercept	0.81 (0.73)			0.80 (0.70)		
Age ≥80	0.47 (0.26)	1.59 (0.96-2.64)	0.071	0.46 (0.25)	1.58 (0.97-2.59)	0.067
Glasgow coma scale (per point increase)	-0.20 (0.05)	0.82 (0.74-0.90)	<0.001	-0.20 (0.05)	0.82 (0.75-0.90)	<0.001
Infratentorial ICH location	0.44 (0.39)	1.55 (0.73-3.33)	0.256	0.41 (0.38)	1.51 (0.72-3.17)	0.272
ICH volume (per ml increase)	0.02 (0.00)	1.02 (1.01-1.03)	<0.001	0.02 (0.00)	1.02 (1.01-1.03)	<0.001
Intraventricular haemorrhage	0.71 (0.27)	2.03 (1.20-3.42)	0.008	0.72 (0.26)	2.05 (1.23-3.40)	0.006
CT SVD score ≥1	1.07 (0.28)	2.92 (1.70-5.02)	<0.001	0.98 (0.26)	2.67 (1.60-4.45)	<0.001

CT = computed tomography. ICH = intracerebral haemorrhage. SVD = small vessel disease.

Table 11.7 Performance measures of the shrunken multivariable logistic regression prediction models for death at one year after the index ICH in the development dataset (n=401) and following internal validation using the same dataset (n=401; 2,000 bootstrap samples)

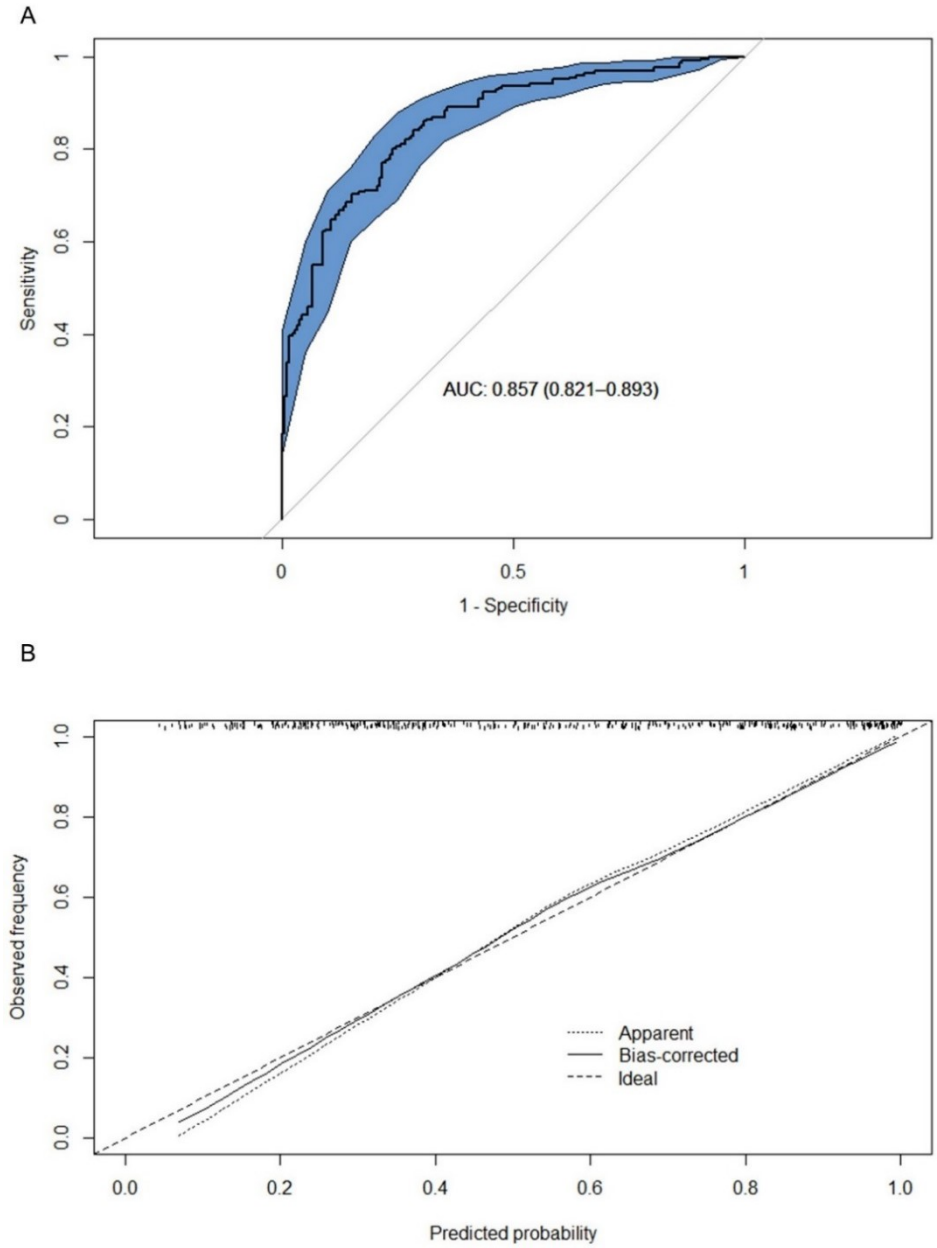
	Development			Internal validation		
	Full model	**ICH score model**	**ICH-SVD score model**	**Full model**	**ICH score model**	**ICH-SVD score model**
Overall						
Brier score	0.15	0.17	0.16	0.16	0.18	0.17
R^2 (Nagelkerke)	0.45	0.38	0.42	0.44	0.38	0.41
Discrimination						
c statistic	0.86	0.82	0.84	0.84	0.81	0.83

ICH = intracerebral haemorrhage. SVD = small vessel disease.

Figure 11.3 Discrimination and calibration measures of the full pre-specified prediction model for death at one year after the index ICH in first-ever SVD-associated ICH following shrinkage.

A. Receiver operating characteristic plot. The AUC is equivalent to the c statistic. The shaded area represents the 95% CI of the AUC based on 2000 bootstrap replicates. The grey line indicates a non-informative AUC of 0.50 for comparison.

B. Calibration plot of model predicted probability versus observed frequency. The dashed grey line indicates ideal calibration, the model's apparent calibration is shown by the dotted grey line. The bias-corrected calibration after internal validation with 2000 bootstrap replicates is shown by the black line. The vertical lines at the top of the plot represent the distribution of model predicted probabilities.

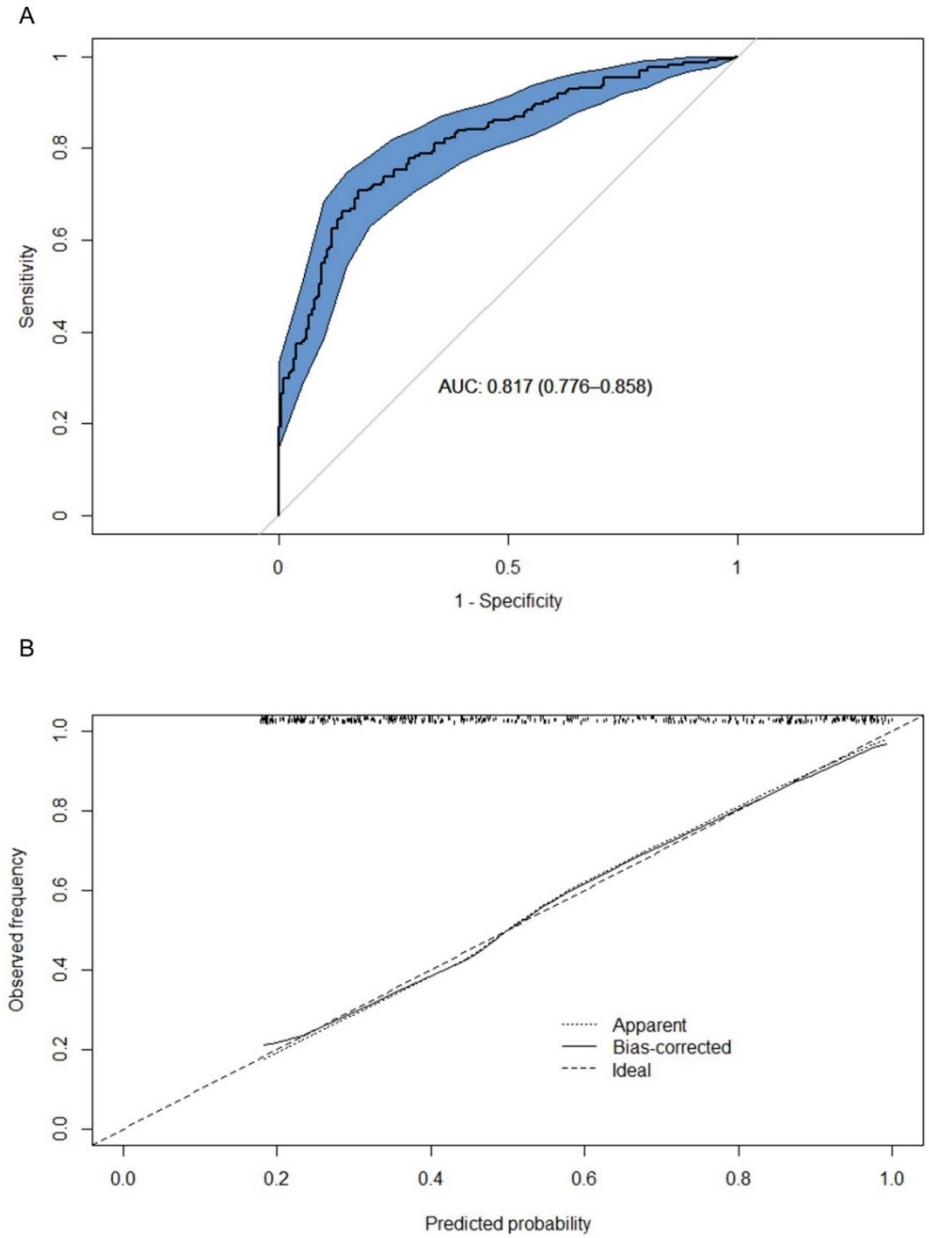


AUC = area under the curve. ICH = intracerebral haemorrhage. SVD = small vessel disease.

Figure 11.4 Discrimination and calibration measures of the ICH score prediction model for death at one year after the index ICH in first-ever SVD-associated ICH following shrinkage.

A. Receiver operating characteristic plot. The AUC is equivalent to the c statistic. The shaded area represents the 95% CI of the AUC based on 2000 bootstrap replicates. The grey line indicates a non-informative AUC of 0.50 for comparison.

B. Calibration plot of model predicted probability versus observed frequency. The dashed grey line indicates ideal calibration, the model's apparent calibration is shown by the dotted grey line. The bias-corrected calibration after internal validation with 2000 bootstrap replicates is shown by the black line. The vertical lines at the top of the plot represent the distribution of model predicted probabilities.

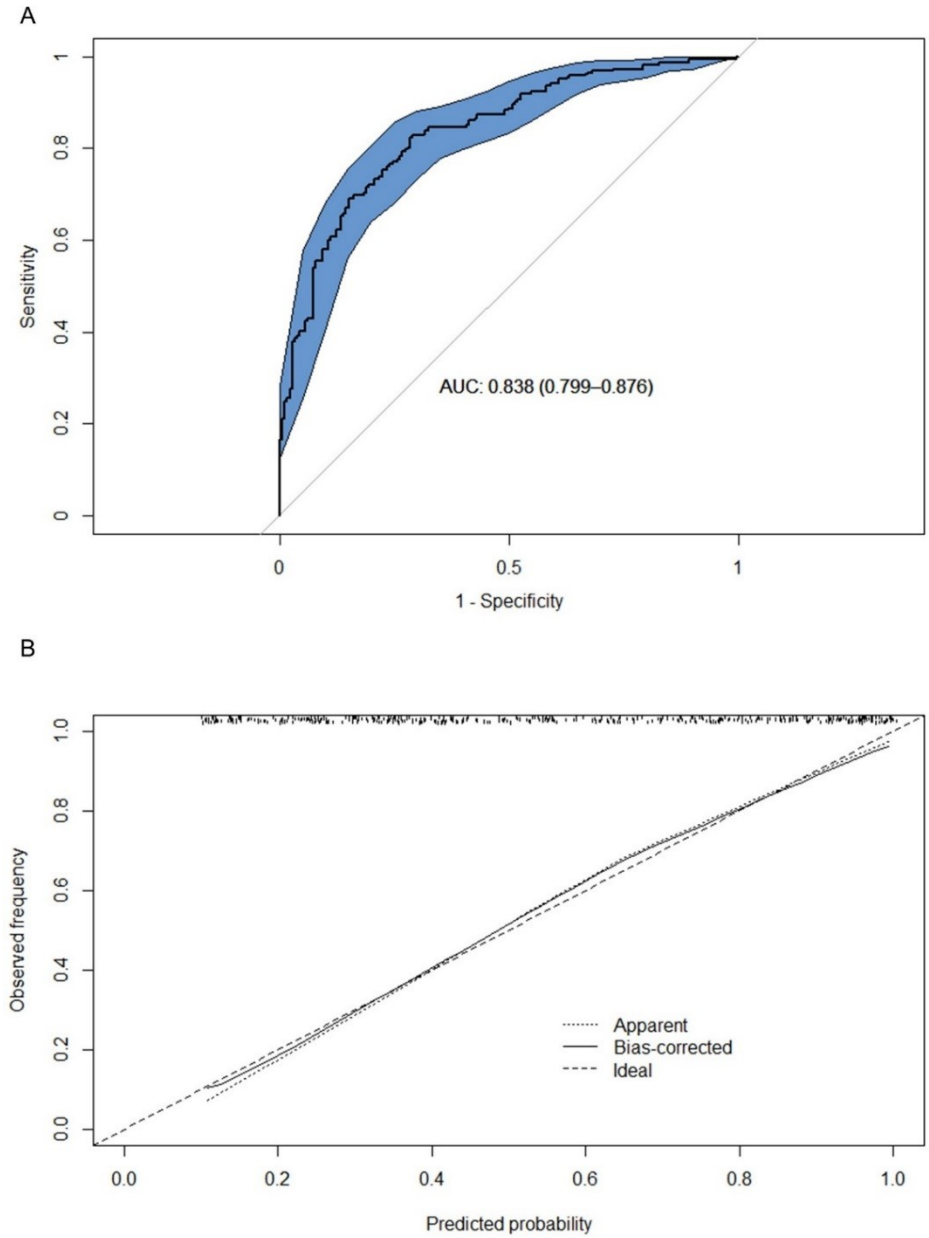


AUC = area under the curve. ICH = intracerebral haemorrhage. SVD = small vessel disease.

Figure 11.5 Discrimination and calibration measures of the ICH-SVD score prediction model for death at one year after the index ICH in first-ever SVD-associated ICH following shrinkage.

A. Receiver operating characteristic plot. The AUC is equivalent to the c statistic. The shaded area represents the 95% CI of the AUC based on 2000 bootstrap replicates. The grey line indicates a non-informative AUC of 0.50 for comparison.

B. Calibration plot of model predicted probability versus observed frequency. The dashed grey line indicates ideal calibration, the model's apparent calibration is shown by the dotted grey line. The bias-corrected calibration after internal validation with 2000 bootstrap replicates is shown by the black line. The vertical lines at the top of the plot represent the distribution of model predicted probabilities.

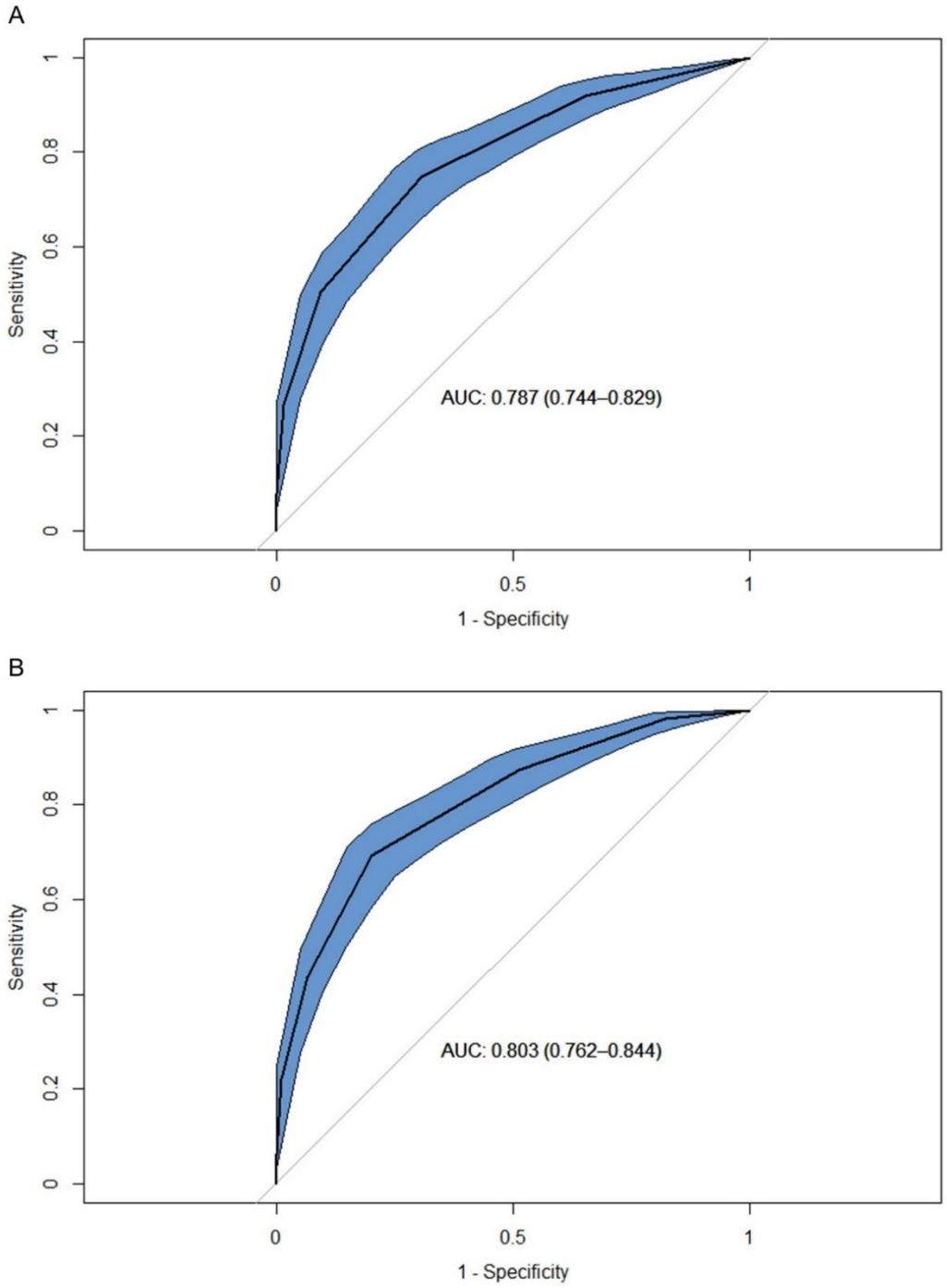


AUC = area under the curve. ICH = intracerebral haemorrhage. SVD = small vessel disease.

Figure 11.6 Receiver operating characteristic plots for death at one year after the index ICH in first-ever SVD-associated ICH.

A. The ICH score. B. The ICH-SVD score.

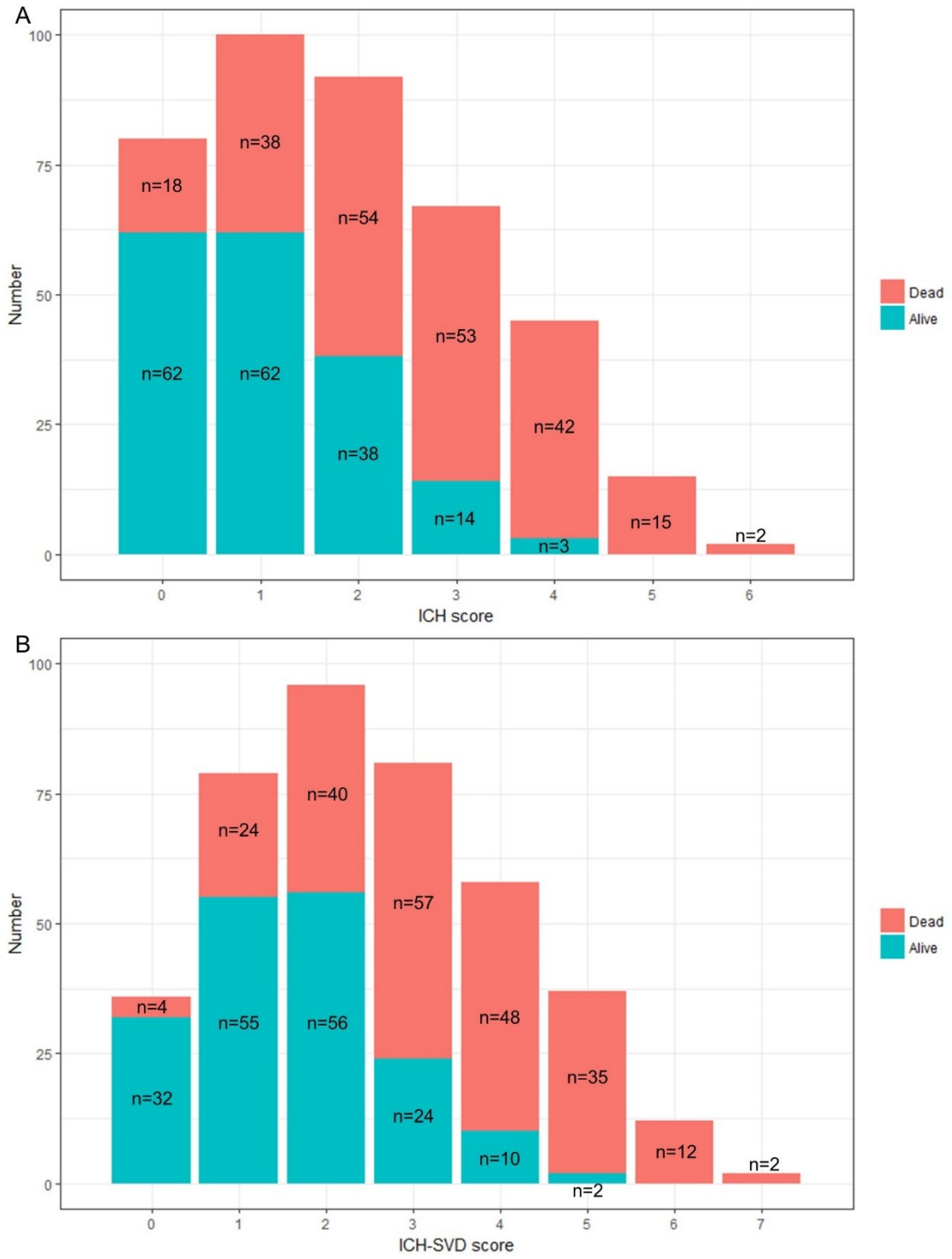
The AUC is equivalent to the c statistic. The shaded area represents the 95% CI of the AUC based on 2000 bootstrap replicates. The grey line indicates a non-informative AUC of 0.50 for comparison.



AUC = area under the curve. ICH = intracerebral haemorrhage SVD = small vessel disease.

Figure 11.7 Bar plots showing the number of patients with first-ever SVD-associated ICH who were dead at one year after their index ICH.

A. The ICH score, B. The ICH-SVD score.



ICH = intracerebral haemorrhage. SVD = small vessel disease.

11.4.5 Prognostic models for death or disability (modified Rankin scale 4-6) at one year after first-ever SVD-associated ICH

I excluded one patient because of missing data for pre-ICH diabetes, two for missing admission GCS score and ten patients with missing one year modified Rankin scales. Therefore, I included 391 patients who had complete data for all predictors and the outcome.

11.4.5.1 Baseline clinical and diagnostic CT brain characteristics

Two hundred and eighty-three patients (72%) were dead or disabled (modified Rankin scale 4-6) at one year after their index ICH while 111 patients were not (modified Rankin scale 0-3). Those who were dead or disabled were older, with a more frequent history of pre-ICH ischaemic stroke, dementia or diabetes, worse pre-ICH modified Rankin scale scores, and lower GCS on admission (Table 11.8). Larger ICH volume and more frequent intraventricular haemorrhage, subarachnoid haemorrhage, and finger-like projections were also associated with death or disability at one year, as was more severe CT SVD score (Table 11.9). There was no association between sex, antiplatelet or anticoagulant drug use at the time of ICH and ICH location and death or disability at one year.

11.4.5.2 Multivariable logistic regression prediction models

Full pre-specified model

The full prediction model for death included 11 pre-specified predictors and is shown in Table 11.10. The variance inflation factor values ranged from 1.05 to 1.76, confirming no evidence of multicollinearity between the predictors.

The shrunken model calculates the predicted probability of death or disability (modified Rankin scale 4-6) at one year after index ICH as follows:

$$Risk\ score = -2.43 + 0.06 * (age) + 0.34 * (male\ sex) - 0.05 * (pre - ICH\ dementia) + 1.14 * (pre - ICH\ diabetes) - 0.50 * (antiplatelet\ use) - 0.17 * (anticoagulant\ use) - 0.24 * (GCS) + 0.50 * (non\ lobar\ ICH\ location) + 0.04 * (ICH\ volume\ ) + 1.17 * (intraventricular\ haemorrhage) + 0.95 * (CT\ SVD\ score \geq 1)$$

$$Predicted\ probability = \frac{1}{1 + exp^{-risk\ score}}$$

The categorical predictor values are 1 when present and 0 when absent, age is the age at the time of the index ICH in years, GCS is the GCS score on hospital admission and ICH volume is the volume of the largest ICH in ml.

ICH score model

The logistic regression prediction model for the variables used in the ICH score is shown in Table 11.11. The variance inflation factor values ranged from 1.01 to 1.09, confirming no evidence of multicollinearity between the predictors.

The shrunken model calculates the predicted probability of death or disability (modified Rankin scale 4-6) at one year after index ICH as follows:

$$Risk\ score = 2.88 + 0.78 * (age) - 0.26 * (GCS) - 0.02 * (infratentorial\ ICH\ location) + 0.03 * (ICH\ volume) + 1.14 * (intraventricular\ haemorrhage)$$

$$Predicted\ probability = \frac{1}{1 + exp^{-risk\ score}}$$

The value of the predictor age is 1 when the age is ≥80 years and 0 when age is <80 years. The other categorical predictor values are 1 when present and 0 when absent. ICH volume is the volume of the largest ICH in ml and GCS is GCS score on hospital admission.

Table 11.8 Baseline clinical features in first-ever SVD-associated ICH patients who were dead or dependent (modified Rankin scale 4-6) at one year after the index ICH versus those who were not (modified Rankin scale 0-3)

	Modified Rankin scale 0-3 (n=111)		**Modified Rankin scale 4-6 (n=283)**		**p value**
Age (years); median (IQR)	72	(60-81)	79	(71-84)	<0.001
Sex					
Female	57	(51)	155	(55)	0.540
Male	54	(49)	128	(45)	
Co-morbidities					
Hypertension	68	(61)	193	(68)	0.175
Ischaemic stroke†	10	(9)	57	(20)	0.008
Transient ischaemic attack†	11	(10)	34	(12)	0.547
Dementia	7	(6)	43	(15)	0.017
Diabetes†	6	(5)	39	(14)	0.018
Atrial fibrillation†	23	(21)	64	(23)	0.671
Myocardial infarction	7	(6)	30	(11)	0.186
Hyperlipidaemia	17	(15)	53	(19)	0.417
Smoking status‡					
Current	24	(25)	61	(23)	
Ex-smoker	31	(32)	97	(37)	0.627
Never	43	(44)	104	(40)	
Pre-ICH modified Rankin scale					
0	62	(56)	65	(23)	
1	22	(20)	59	(21)	
2	16	(14)	71	(26)	
3	11	(10)	66	(24)	
4	0	(0)	14	(5)	
5	0	(0)	3	(1)	
Pre-ICH modified Rankin scale; median (IQR)	1	(1-2)	3	(2-4)	<0.001
Medications on admission					
Antiplatelet drug(s)	41	(37)	129	(46)	0.119
Anticoagulant drug(s)	14	(13)	38	(13)	0.830
Antihypertensive drug(s)	54	(49)	143	(51)	0.737
Admission GCS score; median (IQR)*	15	(14-15)	12	(8-14)	<0.001

Data are n (%), median (IQR) or mean (SD). † data missing in 1 patient . * data missing in 2 patients. ‡ data missing in 34. GCS = Glasgow coma scale. ICH = intracerebral haemorrhage. SVD = small vessel disease.

Table 11.9 Non-contrast brain CT features in first-ever SVD-associated ICH patients who were dead or dependent (modified Rankin scale 4-6) at one year after the index ICH versus those who were not (modified Rankin scale 0-3)

	Modified Rankin scale 0-3 (n=111)		Modified Rankin scale 4-6 (n=283)		p value
ICH location					
Lobar	54	(49)	139	(49)	
Deep	42	(38)	108	(38)	0.978
Infratentorial	15	(14)	36	(13)	
ICH volume (ml); median (IQR)	7	(3-16)	33	(12-76)	<0.001
Intraventricular haemorrhage	19	(17)	169	(60)	<0.001
Subarachnoid haemorrhage	38	(34)	134	(47)	0.018
Subdural haemorrhage	8	(7)	34	(12)	0.164
Finger-like projections	5	(5)	39	(14)	0.009
Number of lacunes; median (IQR)	0	(0-1)	0	(0-1)	0.896
Anterior white matter lucencies					
0	33	(30)	44	(16)	
1	58	(52)	141	(50)	<0.001
2	20	(18)	98	(35)	
Posterior white matter lucencies					
0	54	(49)	68	(24)	
1	21	(19)	71	(25)	<0.001
2	36	(32)	144	(51)	
Central atrophy					
0	38	(34)	62	(22)	
1	70	(63)	166	(59)	<0.001
2	3	(3)	55	(19)	
Cortical atrophy					
0	31	(28)	73	(26)	
1	67	(60)	140	(50)	0.015
2	13	(12)	70	(25)	
CT SVD score					
0	58	(52)	77	(27)	
1	36	(32)	117	(41)	
2	14	(13)	73	(26)	<0.001
3	3	(3)	16	(6)	

Data are n (%), median (IQR) or mean (SD). CT = computed tomography. ICH = intracerebral haemorrhage. SVD = small vessel disease.

Table 11.10 Full pre-specified multivariable logistic regression prediction models for death or disability (modified Rankin scale 4-6) at one year after the index ICH in first-ever SVD-associated ICH

	Original model			Shrunken model		
	β Coefficient (standard error)	Odds ratio (95%CI)	p value	β Coefficient (standard error)	Odds ratio (95%CI)	p value
Intercept	-2.85 (1.67)			-2.43 (1.54)		
Age (per year increase)	0.06 (0.02)	1.06 (1.03-1.10)	<0.001	0.06 (0.01)	1.06 (1.03-1.09)	<0.001
Male sex	0.38 (0.33)	1.46 (0.77-2.76)	0.250	0.34 (0.31)	1.40 (0.77-2.56)	0.274
Pre-ICH dementia	-0.12 (0.54)	0.89 (0.31-2.57)	0.830	-0.05 (0.50)	0.95 (0.36-2.51)	0.920
Pre-ICH diabetes	1.36 (0.55)	3.89 (1.33-11.36)	0.013	1.14 (0.49)	3.14 (1.20-8.21)	0.020
Antiplatelet use at ICH	-0.60 (0.35)	0.55 (0.29-1.09)	0.085	-0.50 (0.33)	0.61 (0.32-1.15)	0.124
Anticoagulant use at ICH	-0.23 (0.50)	0.80 (0.30-2.11)	0.649	-0.17 (0.45)	0.84 (0.35-2.05)	0.708
Glasgow coma scale (per point increase)	-0.25 (0.08)	0.78 (0.67-0.90)	0.001	-0.24 (0.07)	0.79 (0.69-0.91)	0.001
Non lobar ICH location	0.59 (0.33)	1.80 (0.94-3.45)	0.076	0.50 (0.31)	1.65 (0.89-3.04)	0.109
ICH volume (per ml increase)	0.04 (0.01)	1.04 (1.03-1.06)	<0.001	0.04 (0.01)	1.04 (1.02-1.05)	<0.001
Intraventricular haemorrhage	1.20 (0.37)	3.33 (1.61-6.89)	0.001	1.17 (0.35)	3.22 (1.63-6.36)	0.001
CT SVD score ≥1	1.03 (0.33)	2.81 (1.48-5.34)	0.001	0.95 (0.31)	2.60 (1.42-4.76)	0.002

CT = computed tomography. ICH = intracerebral haemorrhage. SVD = small vessel disease.

ICH-SVD score model

I combined the CT SVD score predictor with the other variables from the ICH score to create the ICH-SVD score logistic regression prediction model (Table 11.12). The variance inflation factor values ranged from 1.04 to 1.13, confirming no evidence of multicollinearity between the predictors.

The shrunken model calculates the predicted probability of death or disability (modified Rankin scale 4-6) at one year after index ICH as follows:

$$Risk\ score = 1.97 + 0.56 * (age) - 0.24 * (GCS) - 0.04 * (infratentorial\ ICH\ location) + 0.03 * (ICH\ volume) + 1.14 * (intraventricular\ haemorrhage) + 1.17 * (CT\ SVD\ score \geq 1)$$

$$Predicted\ probability = \frac{1}{1 + exp^{-risk\ score}}$$

The value of the predictor age is 1 when the age is ≥80 years and 0 when age is <80 years. The other categorical predictor values are 1 when present and 0 when absent. ICH volume is the volume of the largest ICH in ml and GCS is GCS score on hospital admission.

11.4.5.3 Performance of the prediction models

The performance measures of the models are summarised in Table 11.13. The full pre-specified model showed good discrimination (c statistic 0.89, 95%CI 0.86-0.92) and good calibration (Figure 11.8). The ICH score model showed good discrimination (c statistic 0.85, 95%CI 0.82-0.89) and no evidence of miscalibration (Figure 11.9). The ICH-SVD score model also showed good discrimination (c statistic 0.87, 95%CI 0.84-0.90) and calibration (Figure 11.10). The AIC was best for the full pre-specified model (311.9), followed by the ICH-SVD model (323.6) then the ICH score model (338.5).

Internal validation resulted in small optimism-adjusted differences in the overall performance measures and discrimination for all three models (Table 11.13), with no evidence of poor calibration (Figure 11.8 to Figure 11.10).

Table 11.11 ICH score multivariable logistic regression prediction models for death or disability (modified Rankin scale 4-6) at one year after the index ICH in first-ever SVD-associated ICH

	Original model			Shrunken model		
	β Coefficient (standard error)	Odds ratio (95%CI)	p value	β Coefficient (standard error)	Odds ratio (95%CI)	p value
Intercept	3.04 (1.11)			2.88 (1.03)		
Age ≥80	0.80 (0.29)	2.24 (1.27-3.95)	0.006	0.78 (0.28)	2.19 (1.25-3.82)	0.006
Glasgow coma scale (per point increase)	-0.27 (0.08)	0.76 (0.66-0.89)	<0.001	-0.26 (0.07)	0.77 (0.67-0.89)	<0.001
Infratentorial ICH location	-0.01 (0.42)	0.99 (0.44-2.23)	0.975	-0.02 (0.41)	0.98 (0.44-2.17)	0.952
ICH volume (per ml increase)	0.03 (0.01)	1.03 (1.02-1.04)	<0.001	0.03 (0.01)	1.03 (1.01-1.04)	<0.001
Intraventricular haemorrhage	1.14 (0.32)	3.12 (1.67-5.84)	<0.001	1.14 (0.31)	3.14 (1.70-5.78)	<0.001

ICH = intracerebral haemorrhage. SVD = small vessel disease.

Table 11.12 ICH-SVD score multivariable logistic regression prediction models for death or disability (modified Rankin scale 4-6) at one year after the index ICH in first-ever SVD-associated ICH

	Original model			Shrunken model		
	β Coefficient (standard error)	**Odds ratio (95%CI)**	**p value**	**β Coefficient (standard error)**	**Odds ratio (95%CI)**	**p value**
Intercept	2.02 (1.11)			1.97 (1.03)		
Age ≥80	0.56 (0.30)	1.76 (0.97-3.18)	0.063	0.56 (0.30)	1.74 (0.98-3.11)	0.060
Glasgow coma scale (per point increase)	-0.25 (0.07)	0.78 (0.67-0.90)	0.001	-0.24 (0.07)	0.79 (0.69-0.90)	0.001
Infratentorial ICH location	-0.02 (0.44)	0.98 (0.42-2.30)	0.959	-0.04 (0.42)	0.96 (0.42-2.21)	0.924
ICH volume (per ml increase)	0.03 (0.01)	1.03 (1.02-1.05)	<0.001	0.03 (0.01)	1.03 (1.02-1.04)	<0.001
Intraventricular haemorrhage	1.14 (0.33)	3.14 (1.64-6.03)	0.001	1.14 (0.32)	3.14 (1.67-5.90)	<0.001
CT SVD score ≥1	1.25 (0.30)	3.50 (1.93-6.35)	<0.001	1.17 (0.29)	3.23 (1.82-5.71)	<0.001

CT = computed tomography. ICH = intracerebral haemorrhage. SVD = small vessel disease.

11.4.6 ICH score versus the ICH-SVD score

The ICH score had a c statistic of 0.81 (95%CI 0.77-0.85). The addition of the predictor CT SVD ≥1 (ICH-SVD score) led to a small increase in the c statistic to 0.83 (95%CI 0.79-0.87) (Figure 11.11).

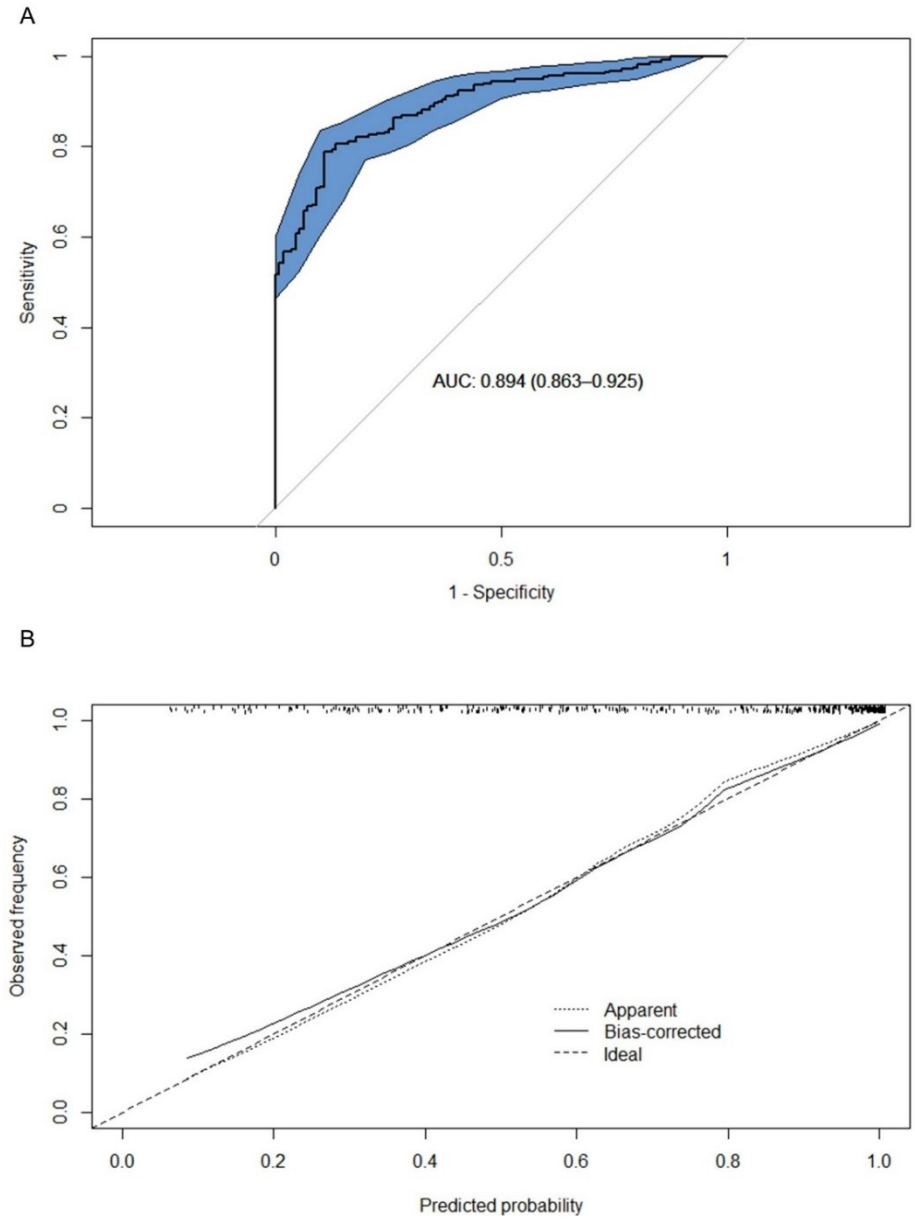
The percentage of patients who were dead or disabled at one year after the index ICH increased with both the ICH score and ICH-SVD (Figure 11.12). Thirty-eight percent (95%CI 28-49%) of patients with an ICH score of 0 were dead or disabled at one year, whereas 94% (85-98%) with an ICH score of 3 and 100% (95%CI 94-100%) with an ICH score of 4, 5 or 6 were dead or disabled.

Twenty-four percent (95%CI 12-40%) of patients with an ICH-SVD score of 0 were dead or disabled at one year after their index ICH, whereas 96% (95%CI 88-99%) with an ICH-SVD score 4 and 100% (95%CI 93-100%) with an ICH-SVD score of 5, 6 or 7 were dead or disabled.

Figure 11.8 Discrimination and calibration measures of the full pre-specified prediction model for death or disability (modified Rankin scale 4-6) at one year after the index ICH in first-ever SVD-associated ICH following shrinkage.

A. Receiver operating characteristic plot. The AUC is equivalent to the c statistic. The shaded area represents the 95% CI of the AUC based on 2000 bootstrap replicates. The grey line indicates a non-informative AUC of 0.50 for comparison.

B. Calibration plot of model predicted probability versus observed frequency. The dashed grey line indicates ideal calibration, the model's apparent calibration is shown by the dotted grey line. The bias-corrected calibration after internal validation with 2000 bootstrap replicates is shown by the black line. The vertical lines at the top of the plot represent the distribution of model predicted probabilities.

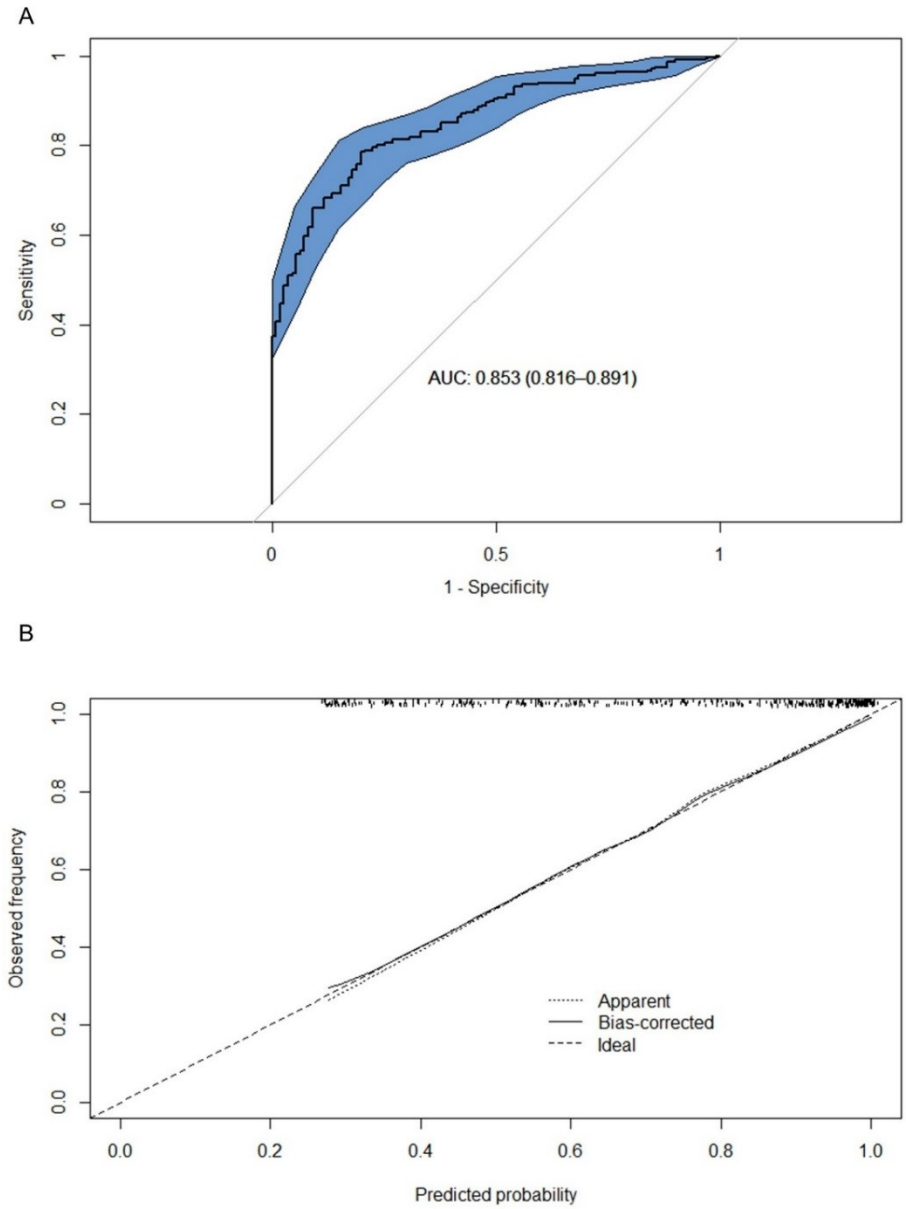


AUC = area under the curve. ICH = intracerebral haemorrhage. SVD = small vessel disease.

Figure 11.9 Discrimination and calibration measures of the ICH score prediction model for death or disability (modified Rankin scale 4-6) at one year after the index ICH in first-ever SVD-associated ICH following shrinkage.

A. Receiver operating characteristic plot. The AUC is equivalent to the c statistic. The shaded area represents the 95% CI of the AUC based on 2000 bootstrap replicates. The grey line indicates a non-informative AUC of 0.50 for comparison.

B. Calibration plot of model predicted probability versus observed frequency. The dashed grey line indicates ideal calibration, the model's apparent calibration is shown by the dotted grey line. The bias-corrected calibration after internal validation with 2000 bootstrap replicates is shown by the black line. The vertical lines at the top of the plot represent the distribution of model predicted probabilities.

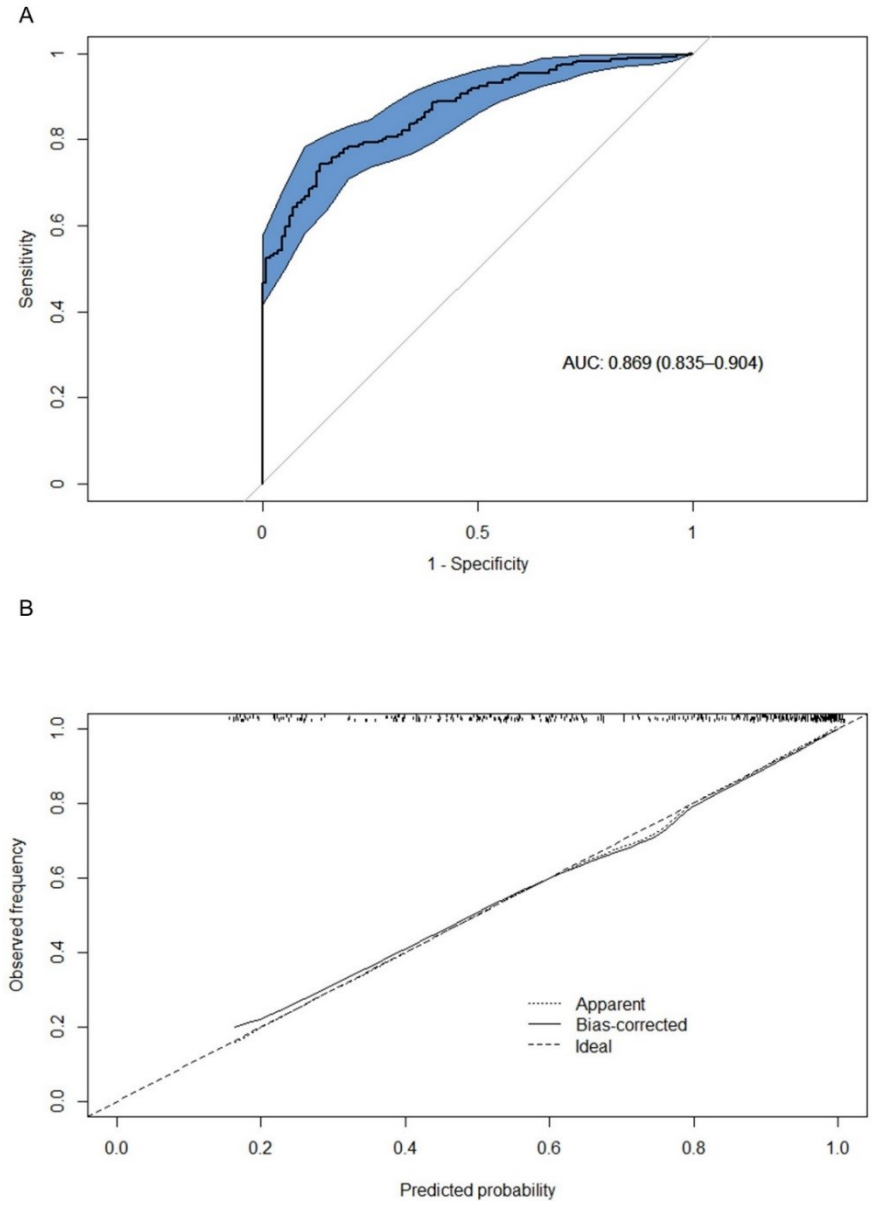


AUC = area under the curve. ICH = intracerebral haemorrhage. SVD = small vessel disease.

Figure 11.10 Discrimination and calibration measures of the ICH-SVD score prediction model for death or disability (modified Rankin scale 4-6) at one year after the index ICH in first-ever SVD-associated ICH following shrinkage.

A. Receiver operating characteristic plot. The AUC is equivalent to the c statistic. The shaded area represents the 95% CI of the AUC based on 2000 bootstrap replicates. The grey line indicates a non-informative AUC of 0.50 for comparison.

B. Calibration plot of model predicted probability versus observed frequency. The dashed grey line indicates ideal calibration, the model's apparent calibration is shown by the dotted grey line. The bias-corrected calibration after internal validation with 2000 bootstrap replicates is shown by the black line. The vertical lines at the top of the plot represent the distribution of model predicted probabilities.



AUC = area under the curve. ICH = intracerebral haemorrhage. SVD = small vessel disease.

Table 11.13 Performance measures of the multivariable logistic regression prediction models for death or disability (modified Rankin scale 4-6) at one year after the index ICH in the development dataset (n=391) and following internal validation using the same dataset (n=391; 2,000 bootstrap samples)

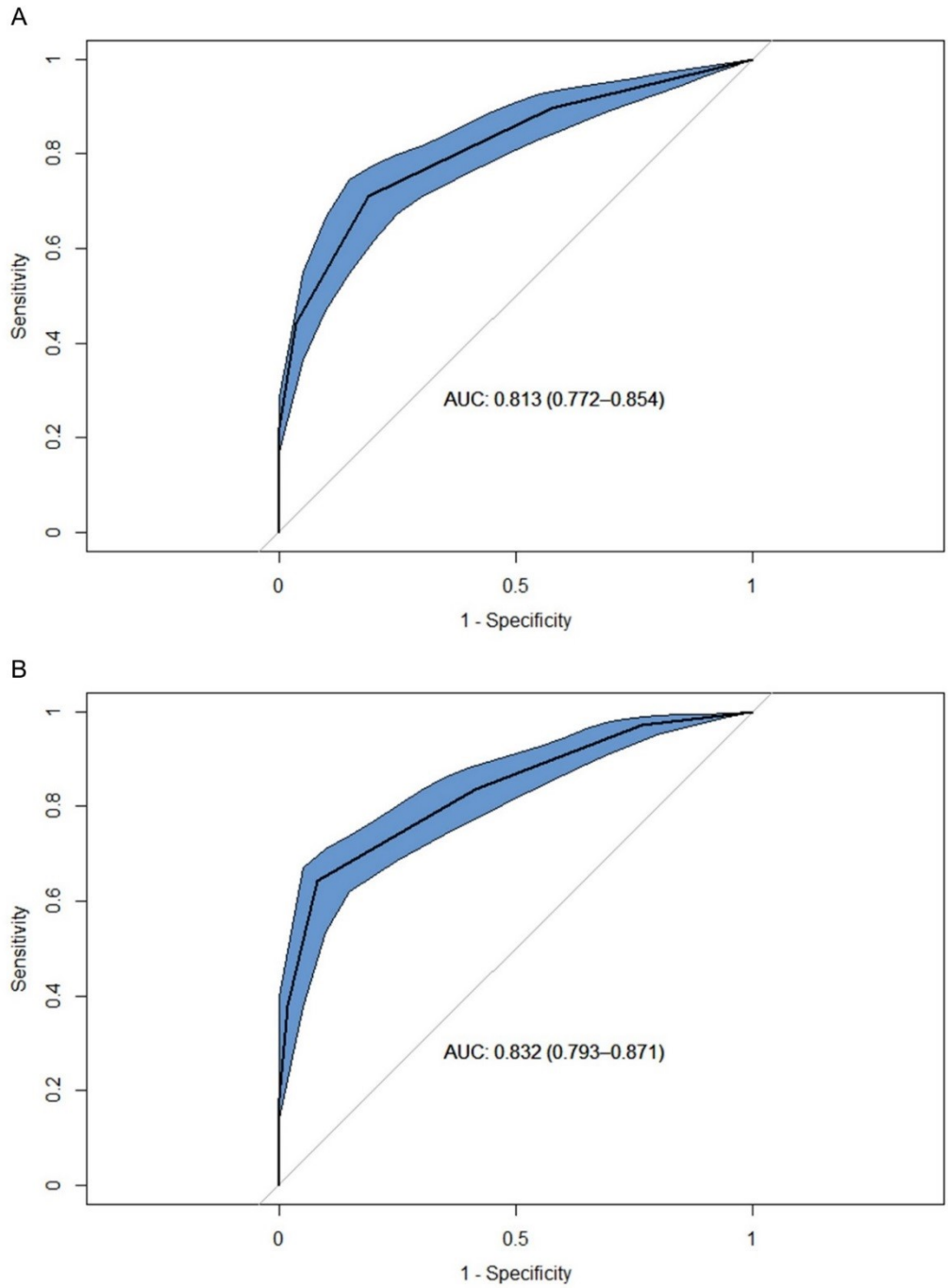
	Development			Internal validation		
	Full model	**ICH score model**	**ICH-SVD model**	**Full model**	**ICH score model**	**ICH-SVD model**
Overall						
Brier score	0.12	0.14	0.13	0.13	0.14	0.14
R^2 (Nagelkerke)	0.52	0.43	0.47	0.50	0.42	0.46
Discrimination						
c statistic	0.89	0.85	0.87	0.88	0.85	0.86

ICH = intracerebral haemorrhage. SVD = small vessel disease

Figure 11.11 Receiver operating characteristic curves for death or disability (modified Rankin scale 4-6) at one year after the index ICH in first-ever SVD-associated ICH.

A. The ICH score and B. The ICH-SVD score.

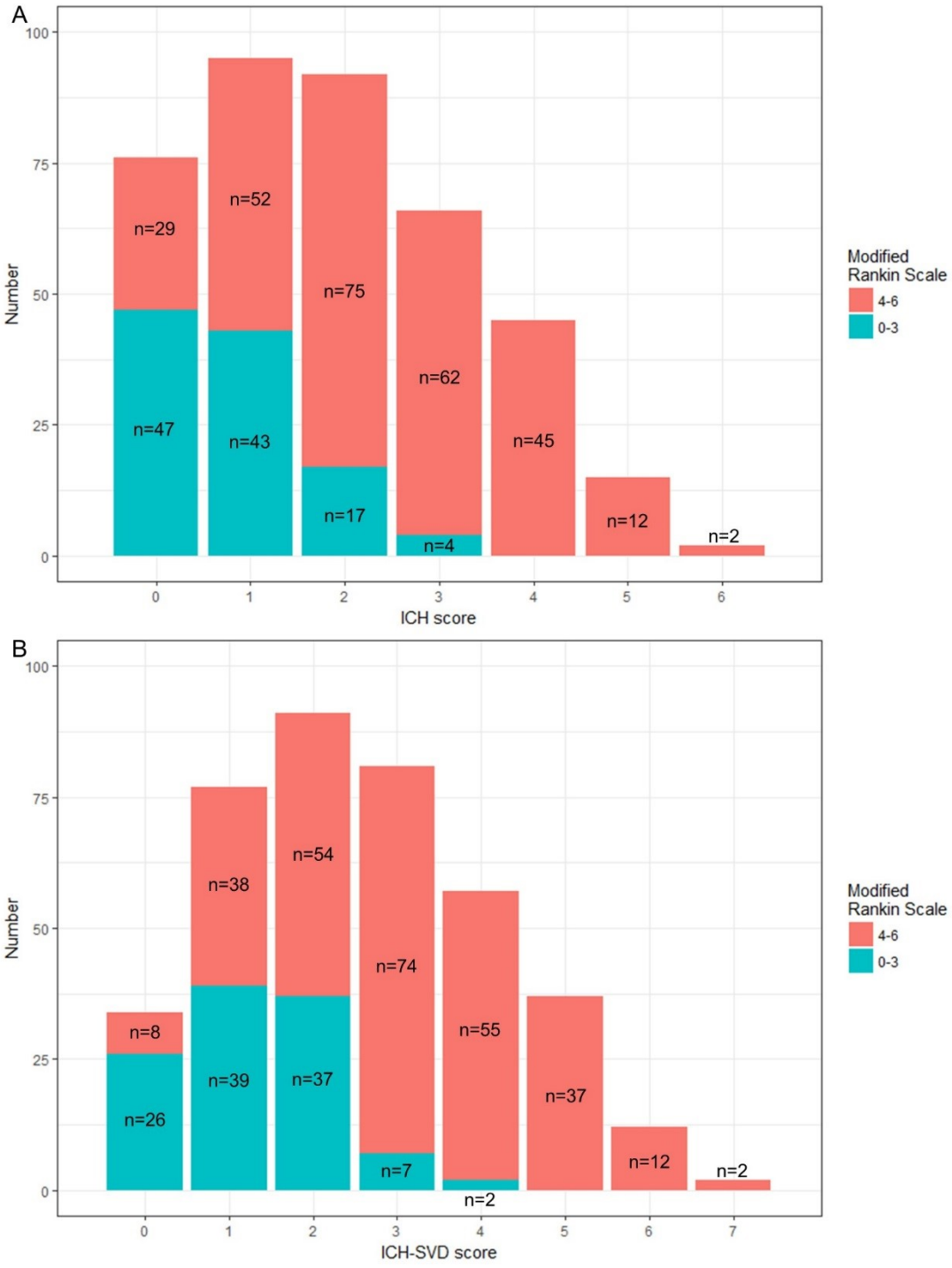
The AUC is equivalent to the c statistic. The shaded area represents the 95% CI of the AUC based on 2000 bootstrap replicates. The grey line indicates a non-informative AUC of 0.50 for comparison.



AUC = area under the curve. ICH = intracerebral haemorrhage. SVD = small vessel disease.

Figure 11.12 Bar plots showing the number of patients with first-ever SVD-associated ICH who were dead or disabled (modified Rankin scale 4-6) at one year after their index ICH.

A. The ICH score and B. The ICH-SVD score.



ICH = intracerebral haemorrhage. SVD = small vessel disease.

11.5 Discussion

11.5.1 Main findings

- 42% of patients with first-ever SVD-associated ICH died within 30 days of their index ICH whilst 56% were dead by one year.
- Death at one year after the index ICH:
    - Increasing age, male sex, decreasing admission GCS, increasing ICH volume, intraventricular haemorrhage and a CT SVD≥1 were independently associated with death at one year.
    - The full pre-specified logistic regression prediction model had the best fit (AIC 407.8) and discrimination (c statistic 0.86) for death at one year.
    - The discrimination of the ICH score was slightly improved by including the CT SVD score (ICH score c statistic 0.79; ICH-SVD score c statistic versus 0.80). This occurred through better stratification of patients at low risk of death (23% with ICH score 0 died within a year compared with 11% with ICH-SVD score 0).
- Death or disability at one year after the index ICH:
    - Increasing age, pre-ICH history of diabetes, decreasing admission GCS, increasing ICH volume, intraventricular haemorrhage and a CT SVD≥1 were independently associated with death or disability at one year.
    - The full pre-specified logistic regression prediction model had the best fit (AIC 311.9) and discrimination (c statistic 0.89) for death or disability at one year.
    - The inclusion of the CT SVD score into the ICH score slightly improved discrimination (ICH score c statistic 0.81; ICH-SVD score c statistic versus 0.83). The ICH-SVD score resulted in better stratification of patients at low risk of death or disability (38% with ICH score 0 were dead or disabled (modified Rankin scale 4-6) at a year compared to 24% with ICH-SVD score 0).

11.5.2 Strengths of the studies

I performed and reported the study according to the TRIPOD guidelines for multivariable prediction models.[332] Important strengths are:

- I reduced selection bias by using data from LATCH, a prospective community-based cohort, which used multiple overlapping sources of case ascertainment. The data is, therefore, representative of a relatively large, contemporary UK population of SVD-associated ICH.
- I only included patients with first-ever ICH to provide a standard inception point.
- I minimised information bias for CT predictor assessment by standardising imaging format and rating the diagnostic non-contrast brain CT using a standardised pro forma. I performed the ratings masked to clinical and outcome data.
- Patients were followed up using multiple sources of data for the outcomes of interest. The completeness of follow-up was very high (>98%).
- The amount of missing predictor and outcome data was low.
- I used clinically relevant, pre-specified outcomes of death and death or disability at one year after the index ICH.
- To minimise overfitting, I pre-specified the predictors for the logistic regression models based on evidence from the literature[151, 244] and restricted the number of predictors to ensure there were at least ten outcomes per predictor in all models. I used penalised maximum likelihood estimation to shrink my prediction models to reduce optimism.[334, 396, 398] I performed internal validation with bootstrapping to account for optimism in the performance of the models[334, 340] and reported the relevant apparent and optimism-adjusted measures of model performance.
- I compared the prediction models against a logistic regression model based on the ICH score,[244] the most widely used prognostic tool for ICH.[375]

- I performed a pragmatic update to the original ICH score by including the CT SVD score (ICH-SVD score) and compared this against the original ICH score.

11.5.3 Limitations of the studies

GCS was assessed by the clinical team caring for the patient, which may introduce variability in its assessment. However, the clinicians in the emergency departments are experienced in assessing GCS, and GCS is known to be reliably used by experienced practitioners.[400] Also, this approach reflects real life clinical practice, making the prediction models more clinically applicable.

The modified Rankin scale at one year after the index ICH was obtained from a postal questionnaire sent to the patients' GPs. The GP may not have specifically assessed the patient for this purpose, which can influence the accuracy of the modified Rankin scale.[401] The modified Rankin scale has only moderate inter-rater agreement, even by experienced researchers.[402] The inter-observer agreement may be lower amongst GPs. These factors will affect the accuracy of the modified Rankin outcome data. Also, GPs were not masked to the presence or absence of the predictors I included in the models. However, they were not aware of this study at the time of their assessment.

The clinical treatment decisions for patients may have been influenced by some of the predictors I included in the models. Both do not attempt resuscitation orders and withdrawal of active care are known predictors of death in those with ICH considered to have a poor prognosis ICH,[403-405] whereas physical therapy and rehabilitation improves functional outcome in ICH.[406] While there were no local guidelines for the use of these interventions during the study period, their use may have been influenced by some of the predictors included in the models, such as age, admission GCS and ICH volume.[403-405] Therefore, the measured outcomes are likely to result from a combination of the direct effects of the predictors and the indirect effect of do not attempt resuscitation orders, withdrawal of active

care[407, 408] - the so-called self-fulfilling prophecy in ICH,[403] and the provision of rehabilitation. Previous studies have shown that prediction models underestimate adverse outcomes in those with early do not attempt resuscitation or withdrawal of active care orders and overestimate them in those without such orders.[408-410] It is difficult to account for this intervention effect bias in predictive modelling. One option is to include the intervention in the prediction model,[407] but this can only be done if the intervention occurs before the follow-up period.[333] An alternative solution is to develop a model in a cohort who received a maximum level of care.[376, 389] However, in my models I wanted to predict the outcome at the time of ICH diagnosis, before do not attempt resuscitation orders and withdrawal of active care are made. This is because the likely prognosis of a patient is important in the decision making for these interventions.[371, 392]

I excluded patients with an ICH secondary to an underlying cause other than SVDs from my studies. However, this information is not known for all patients at the time of initial presentation when the prognostic models might be used. Therefore, to determine their clinical utility, it would be useful to assess the prognostic value of these models in all ICH patients who did not have an underlying cause, such as arteriovenous malformation, aneurysm, venous sinus thrombosis or tumour, identified at the time of initial presentation, regardless of whether one was later identified.

11.5.4 Study findings

11.5.4.1 Survival after ICH

Most of the deaths in ICH occur early after the index event. The 30-day case fatality for patients with first-ever SVD-associated ICH in LATCH was 42% (95%CI 37-46%, Figure 11.1), which is similar to the early ICH case fatality of approximately 40% in a systematic review of population-based studies in high-income countries.[133] The early case fatality in ICH has not improved for several decades.[133]

ICH case fatality continues to increase beyond 30 days. In LATCH, the one-year case fatality rate in first-ever SVD-associated ICH was 56% (95%CI 51-

60%, Figure 11.1), while five-year fatality was 75% (95%CI 71-80%). These figures are similar to the pooled estimates for one-year fatality (54%, 95%CI 51-57%) from nine population-based studies and five-year fatality (71%, 95%CI 67-74%) based on three population-based studies.[151]

In line with previous studies, I found that increasing age, decreasing admission GCS, increasing ICH volume and the presence of intraventricular haemorrhage were independently associated with death at one year.[151] The association between other variables, such as sex and pre-ICH anticoagulant drug use and long term death after ICH is inconsistent.[151] I found male sex was independently associated with death at one year. There was no association between pre-ICH anticoagulant use and death. Some studies have shown pre-ICH anticoagulant drug use is associated with three month mortality, which may relate to its association with haematoma growth.[411] Few studies have assessed the associations of death beyond three months,[151] with one study showing no independent association between anticoagulant use and death three years after ICH.[412]

11.5.4.2 External validation of the ICH score for predicting death and functional outcome at one year after the index ICH

The ICH score[244] is the most widely used outcome prediction tool in ICH.[375] It was developed in 2001 to predict 30-day fatality in ICH and is a simple, pragmatic tool which uses five categorical variables (age, admission GCS, ICH location, ICH volume and intraventricular extension). The ICH score has been widely validated for short term fatality, showing good diagnostic accuracy across a variety of settings.[245, 375, 377-384]

Validation of the ICH score for predicting longer-term fatality is less comprehensive. Two hospital-based studies showed that the ICH score had moderate to good discrimination for 90-day fatality (c statistic 0.74-0.86).[380, 385] I found that in our prospective community-based LATCH cohort, the ICH score had moderate discrimination for one-year fatality after ICH (c statistic 0.79, 95%CI 0.74-0.83). The ICH score was good at identifying those at very high risk of dying (93% with ICH score 4 died, 100% with ICH score 5 or 6

died). In contrast, it was less effective at identifying those with the lowest chance of dying (23% of patients with an ICH score 0 died).

The ICH score has been validated for functional outcome in a several settings.[380, 385-390] In the studies that assessed the modified Rankin scale at one year after the index ICH, the ICH score showed moderate to good discrimination for death or disability (modified Rankin scale 4-6) (c statistic ranged from 0.77 to 0.81).[386-388] I found that the ICH score had good discrimination for one year death or disability (modified Rankin scale 4-6) (c statistic 0.81, 95%CI 0.77-0.85). Again the ICH score appears good at identifying patients with a very high risk of death or disability (94% with ICH score 3 were dead or disabled, while 100% with ICH score of 4 to 6 were). It was less effective at identifying those with a low chance of death or disability (38% with ICH score 0 were dead or disabled at one-year).

11.5.4.3 Improving predictions of death and functional outcome in ICH

The ICH score shows moderate and good discrimination for death and death or disability at one year respectively. However, the ICH score has several limitations. For example, the methodological approaches used to develop the ICH were suboptimal. The ICH score was derived from a logistic regression model. The sample size was modest, with only 68 events. The authors included 11 variables in their model and used stepwise selection to generate the final model. This approach is known to have many disadvantages, such as unstable predictor selection and overestimated model performance, especially when the number of events is low.[334, 336, 339, 413] There was no shrinkage of the regression coefficients, which would have helped reduce overfitting.[336, 413] The model was not internally validated. Internal validation is particularly useful to establish the stability of predictor selection when a stepwise approach has been used.[334, 340] To derive the ICH score, the authors simplified the final logistic regression model by converting the continuous predictors into categorical predicts. This helps make the score easy to implement, but at the cost of a considerable loss of power. The cut-points derived in their relatively small cohort are also likely to introduce bias.[334, 339, 414] Furthermore, the ICH score was developed nearly 20

years ago. The strength of predictor associations may have changed over that time. Also, new potentially important predictors associated with outcome after ICH have been identified,[151] such as white matter lucencies and other CT features of SVD.[195, 391] Therefore, there is potential to update the ICH score in order to improve its prognostic accuracy.

The most effective method to update a prediction model is to assess and update the logistic regression model it is derived from.[334] Unfortunately, the logistic regression equation used to develop the ICH score was not published in the paper,[244] nor was it available after contacting the corresponding author. Therefore, I decided to generate new prediction models using the optimum approaches for predictor selection and modelling (11.3.6.2), and compare them against logistic regression models based on the variables used in the ICH score logistic regression model.

The full pre-specified logistic regression prediction model for death at one year after the index ICH had a better AIC (407.8), a higher c statistic (0.86) and wider range of model predicted probabilities (0.05 to 1, Figure 11.3) compared with the ICH score logistic regression model (AIC 427.6, c statistic 0.82, predicted probability range 0.18 to 0.99, Figure 11.4). A similar pattern was seen for the outcome of death or disability at one year; the full pre-specified logistic regression prediction model had an AIC of 311.9, c statistic of 0.89 with predictions ranging from 0.05 to 1 (Figure 11.8) compared with the model using the ICH score variable (AIC 338.5, c statistic of 0.85, predicted probability range 0.28 to 1, Figure 11.9).

Many modifications to the ICH score have been described.[245, 382, 393, 415] All of these modifications involved adding or adjusting categorical predictors to the original ICH score. I wanted to perform a similar pragmatic update of the ICH model. I added CT SVD score to the other ICH score variables based on the strength of its independent association with the outcomes. The addition of CT SVD score ≥1 into the ICH score led to a small improvement in the c statistic for death at one year (Figure 11.6) and for death or disability at one year (Figure 11.11). The addition of the CT SVD

score improved predictions in those with the lower predicted risk of poor outcomes.

11.5.5 Clinical implications

Prediction modelling is designed to improve the accuracy and reliability of prognostication beyond clinical judgement. Such information is useful to help guide treatment decisions and inform discussions with the patient and their relatives.[371-373] Well designed prediction models can provide accurate estimates for the overall outcome of a population or cohort. One of the benefits of simple scores, such as the ICH score, is that they are quick and easy to calculate. The main disadvantage is the loss of predictive power.

The best prediction models for death and death or disability in my study were the full pre-specified models, which showed high discrimination and good calibration. These models would require external validation before being considered for use in clinical practice. Also, the practicality of full regression-based models need to be assessed. The ICH score is simple to implement, whereas my models would require the use of an online calculator or an app. This is likely to limit the widespread uptake of such a prediction model.

The addition of the CT SVD score to the ICH score did improve the discrimination for death and death or disability at one year, but the magnitude of the improvement is unlikely to be large enough to warrant the use of the ICH-SVD score over the ICH score in clinical practice.

11.5.6 Future directions

As with many areas in medicine, there are multiple prognostic models in ICH, all aiming to predict similar outcomes.[376] Many have significant methodological limitations, such as small sample size, selection bias, detrimental modelling approaches (dichotomising continuous predictors, stepwise selection, overfitting of models without shrinkage or internal validation) and poor reporting of relevant performance measures.

Future research should aim to improve prognostic modelling in ICH through the use of collaborative, multicentre studies. These should ideally be

prospective, with minimal selection bias and low levels of missing data. The outcomes should be clinically relevant, such as death or disability at one year, and assessed in a standardised fashion, with the assessors being masked to the predictors. The predictors assessed in the models should ideally be pre-specified, and the models shrunk and internally validated using bootstrapping to reduce over-fitting. Relevant performance measures should be reported, such as discrimination and calibration. Robust external validation studies need to be performed to establish the true prognostic accuracy. These should also assess models using different patient groups to determine the generalisability, given the racial/ethnic differences in ICH aetiology and outcome.[416] The regression equation should be published to allow external validation and updating of the model in the future.[334, 336, 376]

There is, however, a concern that use of prognostic tools to inform decisions about do not attempt resuscitation orders and withdrawal of active care can result in a self-fulfilling prophecy.[403-405, 407-409] Therefore, further work is needed to assess the clinical impact of prediction models in ICH. Given the trend to avoid early do not attempt resuscitation and withdrawal of active care orders,[417] and the potential for early deterioration,[418, 419] the value of early reassessment on outcome should be investigated.[420]

Section F. Conclusions

Chapter 12 Conclusions

12.1 Main findings of thesis

12.1.1 Cross-sectional studies of SVD-associated ICH

12.1.1.1 Baseline clinical characteristics, radiological features and APOE genotype

- Between 1st June 2010 and 31st May 2013 the crude incidence of first-ever SVD-associated ICH in LATCH was 20.2 per 100,000 per year (95% CI 18.3 to 22.2).
- The crude incidence of lobar and non-lobar first-ever SVD-associated ICH was similar and increased with age.
- Hypertension was a common co-morbidity regardless of ICH location.
- There were no independent associations between pre-existing co-morbidities and ICH location.
- Larger ICH volumes, multiple simultaneous acute ICHs, subarachnoid haemorrhage and subdural haemorrhage were independently associated with lobar ICHs.
- Intraventricular haemorrhage was independently associated with non-lobar ICHs.
- Only 28% of SVD-associated ICH patients underwent brain MRI as part of routine clinical practice or the LINCHPIN study, and they tended to be younger, have fewer co-morbidities and small ICHs than those who did not have MRI.
- Overall, 16% of patients were classified as probable CAA on the modified Boston criteria, 37% as possible CAA and 47% as no CAA.
- In first-ever SVD-associated ICH patients who had a research MRI, cortical superficial siderosis was more frequent in lobar ICH and deep CMBs were more frequent in non-lobar ICH.
- The frequency of lobar CMBs, the total number of CMBs, and the severity of WMH, atrophy and PVS were similar between lobar and non-lobar ICH.

- In first-ever SVD-associated ICH patients, APOE ε2 possession was independently associated with lobar ICH location while APOE ε4 allele possession showed a borderline independent association

12.1.1.2 Histopathological features

- First-ever SVD-associated ICH patients who had a research brain autopsy were older with more frequent pre-ICH dementia and more severe pre-ICH disability, had larger ICHs and more frequent subarachnoid haemorrhage than the rest of the LATCH cohort.
- Histopathological assessment of parenchymal CAA, meningeal CAA and vasculopathy in the left cerebral hemisphere had excellent sensitivity and specificity (≥97%) compared with the global cerebral assessment.
- The Love et al. parenchymal and meningeal CAA scale in the cerebral lobe affected by ICH had good sensitivity and excellent specificity compared with the global cerebral assessment, regardless of age.
- Vonsattel grade≥2 was 95% sensitive and 79% specific compared with global cerebral CAA assessment, but the specificity decreased with age.
- 98% of non-lobar ICHs had moderate or severe non-CAA SVD.
- 45% of lobar ICHs had mixed moderate or severe CAA and non-CAA SVD, 37% had moderate or severe non-CAA alone, while only 15% had moderate or severe CAA alone.
- Moderate or severe parenchymal CAA was significantly associated with APOE ε4 allele possession.
- There was no occipital predominance for parenchymal CAA, meningeal CAA or vasculopathy, whereas capillary CAA showed an occipital predominance irrespective of age, APOE genotype or Thal phase.

12.1.2 Diagnostic value of SVD imaging biomarkers in SVD-associated ICH

12.1.2.1 Diagnostic test accuracy studies of the original and modified Boston criteria for CAA-associated ICH against a histopathological reference standard

- The probable CAA category in the original Boston criteria showed 55% sensitivity and 60% specificity for histopathologically defined CAA-associated ICH.
- The probable CAA category in the modified Boston criteria was 64% sensitive and 40% specific for histopathologically defined CAA-associated ICH.
- However, the study had a small sample size and selection bias.

12.1.2.2 Diagnostic value of β-amyloid PET in SVD-associated ICH

- 6-CN-flutemetamol labels both perivascular (CAA) and non-vascular β-amyloid in *ex vivo* brain tissue from first-ever SVD-associated ICH participants.
- ^{18}F-flutemetamol MRI-PET scans are difficult to perform in ICH patients.
- Overall visual assessment of flutemetamol PET scans showed almost perfect inter-observer agreement (κ 0.90) and correlated with maximum cortical SUVr with no overlap between positive and negative visual classifications.
- Visual assessment of flutemetamol PET had 86% sensitivity and 77% specificity against the modified Boston criteria.
- Maximum cortical SUVr was significantly higher in CAA-associated ICH versus non-CAA-associated ICH, although there was overlap between the groups.

12.1.2.3 The Edinburgh CT and genetic criteria for lobar ICH associated with CAA: model development and diagnostic test accuracy study

- The CT and APOE diagnostic model of subarachnoid haemorrhage, finger-like projections and APOE ε4 allele possession showed excellent

discrimination and good calibration for histopathologically defined CAA-associated lobar ICH.

- The Edinburgh CT-APOE rule out criteria of neither subarachnoid haemorrhage nor APOE ε4 possession had 100% sensitivity, while the rule in criteria of subarachnoid haemorrhage and either APOE ε4 possession or finger-like projections had 96% specificity.
- The CT-only diagnostic model of subarachnoid haemorrhage and finger-like projections showed good discrimination and excellent calibration for histopathologically assessed CAA-associated lobar ICH.
- The Edinburgh CT-only rule out criteria of no subarachnoid haemorrhage or finger-like projections had 89% sensitivity, while the rule in criteria of subarachnoid haemorrhage and finger-like projections had 100% specificity.

12.1.2.4 External validation studies of the Edinburgh CT-only and CT-APOE diagnostic models and criteria for CAA-associated lobar ICH

- In 146 first-ever lobar ICH participants, the Edinburgh CT-only rule out criteria had 88% sensitivity for histopathologically defined CAA-associated lobar ICH, while the rule in criteria were 84% specific.
- In 65 first-ever lobar ICH participants, the Edinburgh CT-APOE rule out criteria were 94% sensitive for histopathologically defined CAA-associated lobar ICH, but the rule in criteria were only 63% specific.

12.1.2.5 Diagnostic test accuracy studies of the Edinburgh diagnostic criteria for CAA-associated lobar ICH against the modified Boston criteria

- Among 70 LINCHPIN first-ever lobar ICH participants with diagnostic CT and research MRI, the Edinburgh CT-only rule out criteria were 81% sensitive for probable CAA on the modified Boston criteria, while the Edinburgh CT-only rule in criteria were 96% specific.
- Among 58 LINCHPIN first-ever lobar ICH participants with diagnostic CT, research MRI and APOE genotyping, the Edinburgh CT-APOE rule out

criteria were 89% sensitive for probable CAA on the modified Boston criteria, and the Edinburgh CT-APOE rule in criteria were 78% specific.

12.1.3 Prognostic value of CT SVD biomarkers in SVD-associated ICH

12.1.3.1 The association between the Edinburgh diagnostic criteria for CAA-associated lobar ICH and the risk of recurrent ICH

- The relative risk and rate of recurrent ICH were significantly higher in participants with first-ever lobar ICH compared with non-lobar ICH.
- In a meta-analysis of the subdistribution hazard models of 462 LATCH and CROMIS-2 participants with first-ever SVD-associated lobar ICH, high risk participants on the Edinburgh CT-only criteria had a significantly higher risk of recurrent ICH compared with the low-risk group.
- Secondary multivariable analyses assessing the individual components of the Edinburgh CT-only criteria showed that subarachnoid haemorrhage was associated with an increased relative risk and rate of recurrent ICH, while finger-like projections were associated with a non-significant increased risk of recurrent ICH.
- In a meta-analysis pooling 384 LINCHPIN and CROMIS-2-DNA participants with first-ever SVD-associated lobar ICH, participants classified as high risk on the Edinburgh CT-APOE criteria had significantly higher risk and rate of recurrent ICH compared with the low-risk group. CT SVD score of 1, 2 or 3 was also associated with an increased hazard of recurrent ICH and death.
- Secondary multivariable analyses assessing the individual components of the Edinburgh CT-APOE criteria and APOE ε2 genotype showed that APOE ε2 allele possession was independently associated with an increased risk and rate of recurrent ICH. Subarachnoid haemorrhage, finger-like projections and APOE ε4 allele possession were not significantly associated with an increased risk of recurrent ICH.

12.1.3.2 Prediction models for death and disability at one year after first-ever SVD-associated ICH

- 42% of first-ever SVD-associated ICH LATCH patients died within 30 days of their index ICH whilst 56% were dead by one year.
- Increasing age, male sex, decreasing admission GCS, increasing ICH volume, intraventricular haemorrhage and a CT SVD≥1 were independently associated with death at one year.
- The discrimination of the ICH score was significantly improved by including the CT SVD score (ICH score c statistic 0.79; ICH-SVD score c statistic versus 0.80, p=0.048).
- 72% of first-ever SVD-associated ICH LATCH patients were dead or disabled (modified Rankin scale 4-6) at one year after their index ICH.
- Increasing age, pre-ICH history of diabetes, decreasing admission GCS, increasing ICH volume, intraventricular haemorrhage and a CT SVD≥1 were independently associated with death or disability at one year.
- The inclusion of the CT SVD score into the ICH score did not significantly improve discrimination (ICH score c statistic 0.81; ICH-SVD score c statistic versus 0.83, p=0.052).

12.2 Implications for routine clinical practice

The incidence of SVD-associated ICH increases dramatically with age, meaning its overall incidence and economic impact is likely to increase as the population ages. Currently there is a lack of effective acute or specific treatments for SVD-associated ICH highlighted by the high levels of death or disability after ICH. Therefore, better prevention of first-ever and recurrent ICH is likely to be the most promising strategy to decrease its burden.

Hypertension is a common co-morbidity in all SVD-associated ICH, regardless of ICH location. In addition, non-CAA SVD, which is associated with hypertension, is the most common type of SVD on histopathological assessment in SVD-associated ICH, even in lobar ICH. The prevention, early

detection and effective management of hypertension is therefore likely to be key to reduce the impact of SVD-associated ICH.

Non-lobar ICH is almost universally associated with moderate or severe non-CAA SVD. Lobar ICH has more heterogeneous histopathology. Whilst 60% of lobar ICH participants had moderate or severe CAA, 82% had moderate or severe non-CAA-SVD. Therefore, lobar ICH should not be assumes to be CAA-associated, even in the elderly.

Diagnosing CAA-associated ICH is clinically important given the associated increased risk of recurrent ICH and post-stroke dementia. When available, histopathological assessment is the reference standard for diagnosing CAA. The absence of amyloid-β in a cortical biopsy (Vonsattel grade 0) can be used to rule out CAA-associated lobar ICH. The presence of complete replacement of a vessel wall with amyloid-β (Vonsattel grade ≥2) in a cortical biopsy can be used to rule in CAA-associated ICH in those aged under 85 years. Its specificity for CAA-associated ICH decreases in those older than 85 years due to the higher frequency of incidental CAA.

However, cortical biopsy is rarely performed, so neuroimaging is usually used to identify CAA-associated ICH. The MRI-based modified Boston criteria are the most commonly used approach. There are two main drawbacks of these criteria in clinical practice. Firstly, their diagnostic accuracy is unclear in ICH, let alone in non-ICH presentations, as the criteria have never undergone rigorous external validation. I showed the modified Boston criteria had limited sensitivity and specificity for CAA-associated ICH in my small external validation study. And secondly, MRI is difficult to perform in ICH. In my community-based cross-sectional study only 28% of ICH patients were able to have an MRI, and these tended to be younger with less severe ICHs.

Amyloid PET may be more sensitive for CAA than structural neuroimaging, however its diagnostic accuracy for CAA-associated ICH is currently unknown. In addition, it is often difficult to perform in ICH patients due to frailty or co-morbidities.

CT is readily available and the most frequent test to diagnose SVD-associated ICH. The Edinburgh CT-only criteria for CAA-associated lobar ICH showed good sensitivity and specificity CAA-associated lobar ICH in both my development and external validation studies. Therefore, the criteria can be used to help rule out and rule in CAA-associated ICH. This potentially obviates the need for further invasive investigations, such as cortical biopsy, which carries potential complications.

The risk of recurrent ICH is higher in patients with a lobar ICH, and appears highest in lobar ICH with CT features of a CAA-associated ICH and severe SVD. Therefore effective secondary prevention in these groups is particularly important.

Finally, I showed that the ICH score is good pragmatic tool for predicting death and death or disability at one year after ICH, particularly for those with a very low or very high score.

12.3 Implications for future research

12.3.1 Epidemiology

Future epidemiological studies should assess the changing incidence of ICH in high-, middle- and low-income countries and investigate the associations with age, co-morbidities and medication use, such as antithrombotic drugs.

12.3.2 Pathology

Further representative histopathological studies of ICH are needed to investigate the clinical, genetic and histopathological associations of CAA and non-CAA SVDs in ICH. The associations and clinical relevance of "pure" CAA-associated lobar ICH, “pure” non-CAA-associated lobar ICH and mixed SVD-associated lobar ICH would be interesting to investigate.

12.3.3 Diagnostic value of SVD imaging biomarkers

The Edinburgh CT-APOE criteria should be externally validated in a large, unselected group to determine its diagnostic accuracy. It will be important to

assess the diagnostic accuracy of the Edinburgh criteria in non-European or North American ICH patients, in whom the incidence of CAA-associated lobar ICH may differ to those included in the development and external validation studies. The inter-rater agreement of the Edinburgh criteria CT markers should be measured in raters of differing experience. Future studies could aim to improve the Edinburgh diagnostic models and criteria through the assessment of CT, genetic and clinical features for differentiating "pure" CAA-associated lobar ICH, “pure” non-CAA-associated lobar ICH and mixed SVD-associated lobar ICH.

Large, rigorous external validation studies of the diagnostic accuracy of MRI-based SVD biomarkers for CAA-associated ICH are required to establish their diagnostic accuracy. Use of prediction models, based on logistic regression or machine learning approaches, will help develop more reliable diagnostic criteria, particularly as most patients with lobar ICH have mixed CAA and non-CAA SVDs underlying their haemorrhage.

It will be vital to compare the diagnostic accuracy of CT and MRI biomarkers for CAA-associated ICH, to determine whether the diagnostic CT scan can be used to identify which patients will benefit from MRI scanning, access to which is often more limited.

Molecular imaging has the theoretical advantages of better sensitivity and specificity than structural imaging for neurodegenerative diseases, such as SVDs. However, the diagnostic value of amyloid PET imaging in SVD-associated ICH is unclear. Well-designed diagnostic accuracy studies assessing clinically relevant amyloid PET tracers (e.g. ^{18}F-labelled tracers) against a histopathological reference standard are needed. The value of amyloid PET for detecting CAA early in the disease process, before ICH occurs, is difficult to assess as tissue samples are not usually available and the diagnostic accuracy of MRI-based SVD biomarkers in this group is not known. One approach would be to perform longitudinal studies comparing amyloid PET and MRI biomarkers in pre-symptomatic hereditary CAA carriers against non-affected family members.

The current amyloid PET tracers are non-specific amyloid ligands, binding to both perivascular and non-vascular β-amyloid. Non-vascular β-amyloid is a frequent finding in the elderly, even those without dementia. Hence, the development of a specific PET tracer for CAA would be an important step to improve the specificity of PET imaging for CAA.

12.3.4 Prognostic value of SVD imaging biomarkers

The prognostic value of the Edinburgh criteria for predicting risk of recurrent ICH should be studied in further detail, accounting for relevant confounders, such as blood pressure control and antithrombotic drug use.

ICH survivors are at risk of both ischaemic and haemorrhagic future events. Studies assessing the risk of vaso-occlusive and haemorrhagic outcomes in ICH survivors randomised to taking or avoid antithrombotics according to neuroimaging biomarkers of SVDs will be important to help guide treatment decisions.

The association of the Edinburgh criteria and CT SVD score with the development of post-stroke dementia will be interesting to investigate given that CAA-associated ICH is thought to be associated with a higher risk of developing this outcome.

Prognostic tools, such as the ICH score, are useful for predicting short and longer term fatality. These tools are usually based on baseline clinical and radiological features. Early reassessment of some of these features may help improve the predictions and should be investigated.

Section G. References and Appendices

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LINCHPIN CT RATING FORM

LINCHPIN ID: _____ SCAN DATE: _____

READ DATE: _____ RATER INITIALS: _____

IMAGE SERIES: *Significant series (unenhanced 5mm Axial, coronal & sagittal reformats)*

Grade overall image quality: Poor Adequate Good

1.	Is there any sign of acute ischaemic change?	Y	N	IF NO, GO TO Q.2
a.	On which side of the brain is the ischaemia?	R	L	BOTH
2.	Is there any acute parenchymal haemorrhage?	Y	N	IF NO, GO TO Q.8
a.	Is the main haemorrhage most likely Haemorrhagic Transformation of an Infarct or Primary Intracerebral Haemorrhage?	HTI	PICH	IF HTI, GO TO Q.8
3.	Are there multiple sites of acute parenchymal haemorrhage?	Y	N	
a.	Characterize each separate acute parenchymal haemorrhage starting with the largest:			

	1	2	3	4	5
Left/Right/ Central					
Anatomic Location code(s) (site(s) are which the ICH is thought to be centred)					
Deep (D), Lobar (L), Mixed lobar & deep (M), Infratentorial (IT) Uncertain (U)					

Anatomic Location Codes for haemorrhages

LOBAR		**DEEP**	
F	Frontal lobe (not basal ganglia)	BG	Basal ganglia (not thalamus)
TE	Temporal lobe	TH	Thalamus
PA	Parietal lobe	IC	Internal capsule
O	Occipital lobe	EC	External capsule
INFRATENTORIAL		WM	Deep & periventricular WM and corpus callosum
CE	Cerebellum		
BS	Brainstem (Midbrain, pons, medulla)		

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SCAN ID: _____ LINCHPIN CT P2

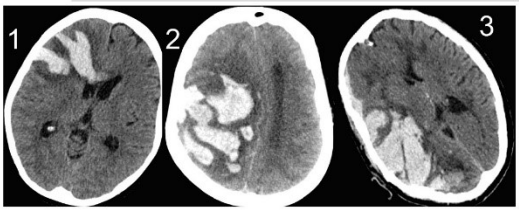
b.	Is there any intraventricular haemorrhage?	Y	N	
c.	Is there any subarachnoid extension?	Y	N	IF YES, GO TO 1D. IF NO, GO TO 1E
d.	If there is subarachnoid extension, is this adjacent to the ICH? Record If distant or both	Y	N	
e.	Is there any subdural extension?	Y	N	
f.	Is there any midline shift or herniation?	Y	N	

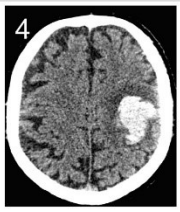
4. Is there a blood/fluid level within any parenchymal haemorrhage? (NOT including an intraventricular fluid level; start with the largest first)

	1	2	3	4	5
Blood or fluid level (Y/N)	☐	☐	☐	☐	☐

5. Describe the border and shape of each separate acute parenchymal haemorrhage starting with the largest first (see examples below)

	1	2	3	4	5
a. Irregular border? (Y/N)	☐	☐	☐	☐	☐
b. Finger-like protrusions? (Y/N)	☐	☐	☐	☐	☐
c. Round/oval? (Y/N)	☐	☐	☐	☐	☐
d. Reaches cortex? (Y/N)	☐	☐	☐	☐	☐





Images 1-3
Irregular border with **finger-like protrusions** to **cortex**

Images 4
Irregular border, reaches **cortex without** finger-like protrusions

Images 5
Regular border **without** finger-like protrusions & **does not** reach cortex

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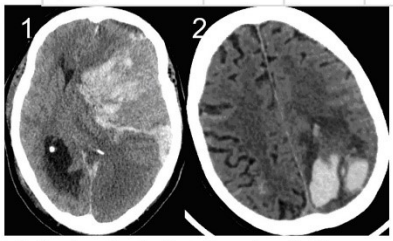
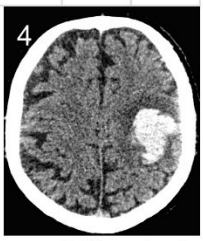
SCAN ID: _____ LINCHPIN CT P3

6.	Describe the density of each acute parenchymal haemorrhage starting with the largest first									
	1		2	3	4	5				
a. Variable density? (Y/N)	☐		☐	☐	☐	☐				
b. Dilute/seeping appearance? (Y/N)	☐		☐	☐	☐	☐				



Variable density & dilute/seeping (1-2)
Blood in any single ICH has variable **density** & appears 'dilute'/seeping into adjacent brain tissue

Variable density (3)
Blood in any single ICH has variable **density** but not 'dilute'



Uniform density (4)
Blood in any single ICH has uniform **homogeneous density**

7.	Characterize the parenchymal haemorrhage(s) listed in 3 further. (Do not include extra-axial haemorrhage in the measurements)			
a.	What is the size of haemorrhage no.1 (mm)? A is largest diameter in the axial plane, B is longest axis perpendicular to A in the axial plane, C is maximum diameter in craniocaudal plane	A	B	C
b.	What is the size of haemorrhage no.2 (mm)?	A	B	C
c.	What is the size of haemorrhage no.3 (mm)?	A	B	C
d.	What is the size of haemorrhage no.4 (mm)?	A	B	C
e.	What is the size of haemorrhage no.5 (mm)?	A	B	C

8.	Are there any **old** vascular lesions?	Y	N	IF NO, GO TO Q.9
a.	Classify the old vascular lesions			

	A	B	C	D	E	F	G			
Code Y/N	☐	☐	☐	☐	☐	☐	☐			

A = old cortical infarct(s) B = old striatocapsular infarct(s) C = old borderzone infarct(s)

D = old lacunes E = old brainstem/cerebellar infarct(s) F = probable old **deep** haemorrhage

G = probable old **lobar** haemorrhage

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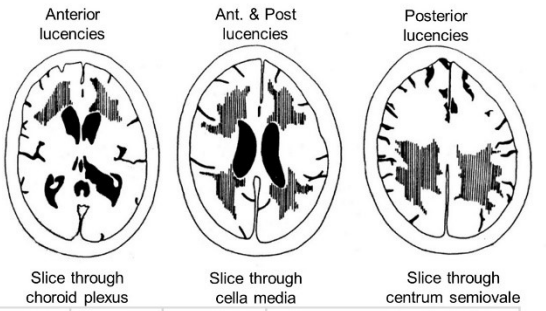
SCAN ID: _____ LINCHPIN CT P4

9.	Classify any PERIVENTRICULAR LUCENCIES		[match "template" below]

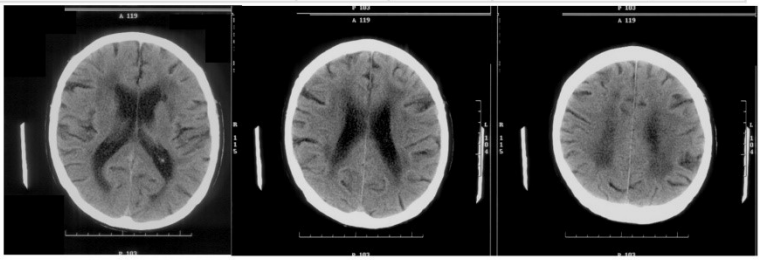
diagram from van Swieten et al. JNNP 1990;53:1080-1083

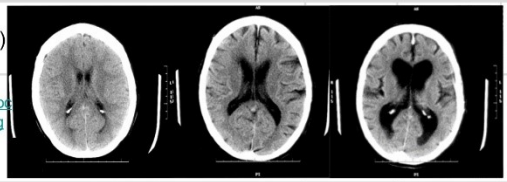
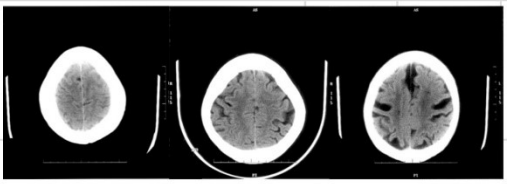


Rating white matter lucency:
0= no lucency
1= lucency restricted to region adjoining ventricles
2= lucency covering entire region from lateral ventricle to cortex

a.	Anterior white matter		0,1,2	Overall ☐
b.	Posterior white matter		0,1,2	Overall ☐

Exemplar:
AWM = 2 PWM = 1
Source:
http://www.sbirc.ed.ac.uk/documents/ctandmr%20reading%20form.pdf



10	Rate any ATROPHY 0 = None. 1 = Moderate 2= Severe		[match "template" below]
a.	Central (deep)		Overall ☐
b.	Cortical		Overall ☐

Source:
http://www.sbirc.ed.ac.uk/documents/ctandmr%20reading%20form.pdf

11	Comments

LINCHPIN CT Rating form v.3 07/04/16 – Derived with permission from IST3/LSR

LINCHPIN: MRI READING FORM

P1

LINCHPIN ID: _____ SCAN DATE: _____

READ DATE: _____ RATER INITIALS: _____

MR Sequences:					
	T1 sag ☐	T1 cor ☐	SWAN ☐
	T2 axial ☐	T2 sag ☐	SWI ☐
	FLAIR ☐	GRE ☐	MRA ☐
	DWI ☐	ADC ☐	MRV ☐

Grade overall image quality: Poor Adequate Good

1.	Is this scan for assessment of the notifying event (*e.g.* detection and/or classification of an acute haematoma)?	Y	N	IF NO, GO TO Q.5
2.	Is there any acute parenchymal haemorrhage?	Y	N	
b.	Is the main haemorrhage most likely Haemorrhagic Transformation of an Infarct or Primary Intracerebral Haemorrhage?	HTI	PICH	IF HTI, GO TO Q.5

3. Characterize each separate acute parenchymal haemorrhage starting with the largest / symptomatic/ most important at number 1. Rank haemorrhages in order of importance.

a.	1	2	3	4	5
Left / Right					
Anatomic Location code (site where ICH is thought to be centred)					
Strictly lobar (L), deep (D), mixed lobar-deep (M), any infratentorial (IT), uncertain (U)					

b.	Is there any intraventricular haemorrhage?	Y	N
c.	Is there any subarachnoid extension?	Y	N
d.	Is there any subdural extension?	Y	N
d.	Is there any midline shift or herniation?	Y	N

Anatomic Location Codes for haemorrhages (use multiple codes if necessary)

LOBAR		**DEEP**	
F	Frontal lobe (not basal ganglia)	BG	Basal ganglia (not thalamus)
TE	Temporal lobe	TH	Thalamus
PA	Parietal lobe	IC	Internal capsule
O	Occipital lobe	EC	External capsule
INFRATENTORIAL		WM	Deep & periventricular WM and corpus callosum
CE	Cerebellum		
BS	Brainstem (Midbrain, pons, medulla)		

Version 2 21/04/17

SCAN ID: _____ P2

4. Characterize the haemorrhage(s) listed in 3a further.

a. What is the size of haemorrhage no.1 (mm)?
A is greatest axial diameter on the largest haemorrhage slice, B is perpendicular to A, C is maximum diameter in craniocaudal plane — A ☐ B ☐ C ☐

b. What is the size of haemorrhage no.2 (mm)? A ☐ B ☐ C ☐

c. What is the size of haemorrhage no.3 (mm)? A ☐ B ☐ C ☐

d. What is the size of haemorrhage no.4 (mm)? A ☐ B ☐ C ☐

e. What is the size of haemorrhage no.5 (mm)? A ☐ B ☐ C ☐

5. Are there any acute infarcts? Y ☐ N ☐ IF NO, GO TO Q.6

a. Classify the acute infarcts

	1	2	3	4	5	6	7	8	9	10
Left / Right	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Vascular location code	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

6. Are there any chronic infarcts or haemorrhages (which are not microbleeds)? Y ☐ N ☐ IF NO, GO TO Q.7

a. Classify the chronic infarcts and haemorrhage (for ICHs use anatomic location codes in 3a)

	1	2	3	4	5	6	7	8	9	10
Left / Right	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Infarct [I] or Haem [H]	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
If lacunar, cavitated Y/N	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Vascular location code	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Vascular Territory Location Codes for infarcts

MCA cortical
1 small cortical
2 basal ganglia
3 subcortical
4 ant half periph MCA
5 post half periph MCA
6 whole peripheral MCA
7 whole periph + lat BG
8 whole MCA territory

Other cortical
9 anterior ACA
10 posterior ACA
11 anterior PCA
12 posterior PCA
13 anterior BZ
14 posterior BZ

Lacunar**
15 int & ext capsules/lent nucleus
16 internal border zone
17 centrum semiovale
18 thalamus
19 brainstem
20 anterior (mainly) borderzone
21 posterior (mainly) borderzone

Cerebellum
22 small cortical
23 <1/2 hemisph.
24 >1/2 hemisph.

Brainstem
25 small (i.e. <1/2 medulla)
26 extensive

1. Record in the following order: MCA cortical > other cortical > lacunar > cerebellum > brainstem
** Defined as a 'CSF-containing cavity' in an appropriate site, measuring 3-20mm in diameter

Version 2 18/02/16

SCAN ID: _____

P3

7	Superficial siderosis?	Y	N	Is if focal (≤3 sulci)?	Y	N	Is it adjacent to an ICH?	Y	N
Side & anatomical location (see 3a)									
Side & vascular territory location (see 6a)									
How many sulci involved									

8. Rate any BASAL GANGLIA MINERAL DEPOSITS [use GRE] Code (0-3): R L

9. Rate any WHITE MATTER HYPERINTENSITIES [FLAIR / T2 most useful]

Fazekas 0/1/2/3

Periventricular R L Overall ☐

Deep (subcortical white matter) R L Overall ☐

10 Rate ATROPHY using normative age template
1 (<25th) 2 (25-50th) 3 (50-75th) 4(75-95th) 5 (>95th) 6 (>>5) [T2 to match template]

a. Deep Overall ☐

b. Cortical Overall ☐

11 Rate any ENLARGED PERIVASCULAR SPACES
0 = None. 1 = ≤ 10. 2 = 11 – 20. 3 = 21 – 40. 4 = > 40. [T2 best]

a. Hippocampus R L Overall ☐

b. Basal ganglia R L Overall ☐

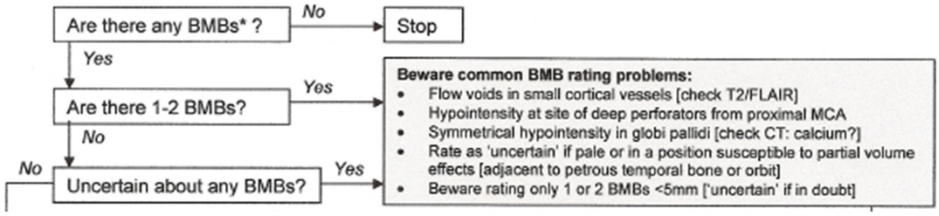
c. Centrum semiovale R L Overall ☐

d. Midbrain R L Overall ☐

Version 2 21/04/17

SCAN ID: _____ P4

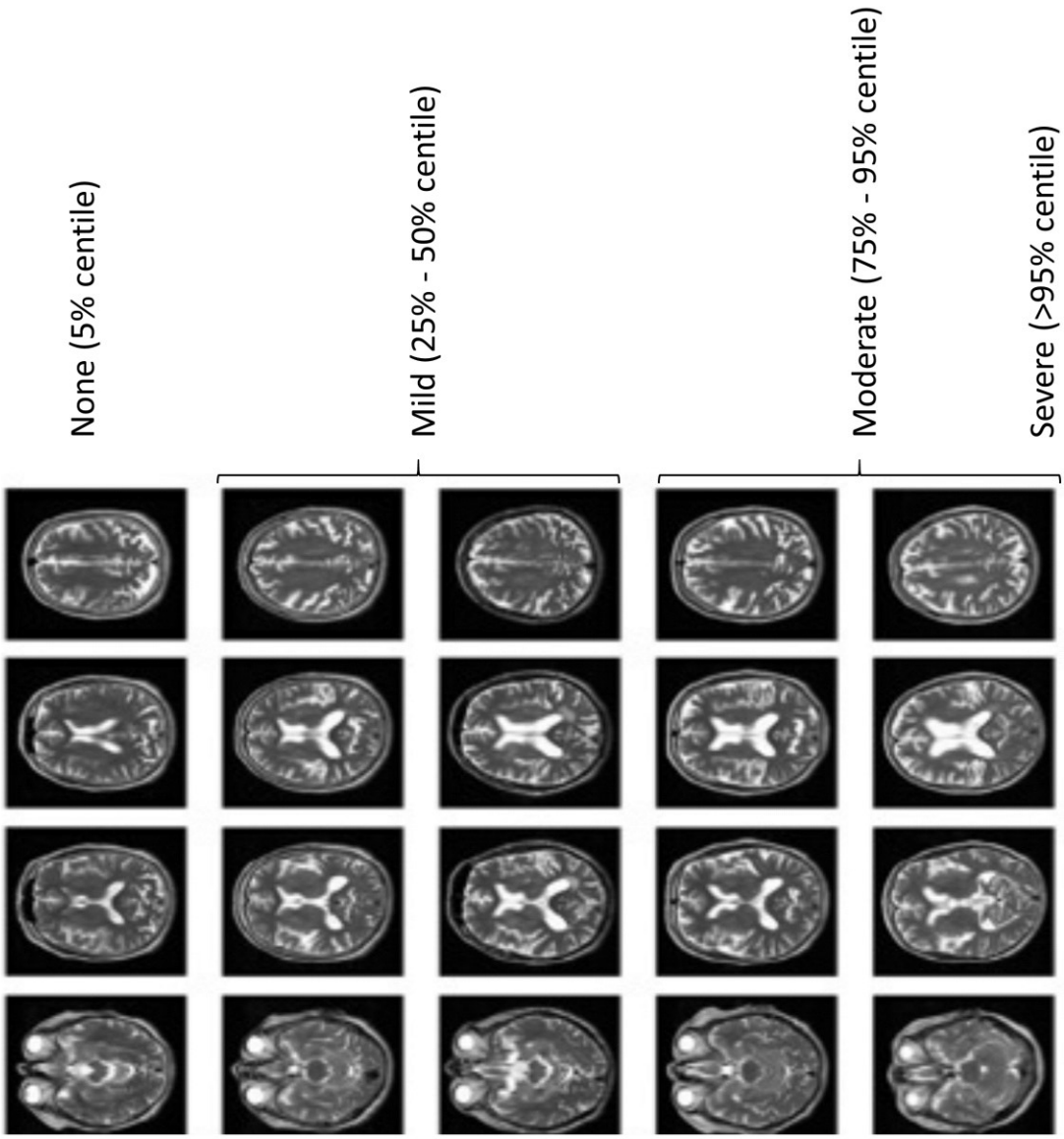
12	Is the T2*/GRE image quality adequate to rate brain microbleeds (BMB), 2-10mm on T2*	Yes →Continue, No → Comments



Quantify BMBs on T2*/GRE about which you are certain:		RIGHT				LEFT			
		Total (GRE)	Total (SWI)	cortical	Sub-cortical	Total (GRE)	Total (SWI)	cortical	Sub-cortical
a.	Frontal lobe	☐	☐	☐	☐	☐	☐	☐	☐
b.	Parietal lobe	☐	☐	☐	☐	☐	☐	☐	☐
c.	Temporal lobe	☐	☐	☐	☐	☐	☐	☐	☐
d.	Occipital lobe	☐	☐	☐	☐	☐	☐	☐	☐
	LOBAR Total	☐	☐	☐	☐	☐	☐	☐	☐
e.	Basal ganglia grey matter	☐	☐			☐	☐		
f.	Internal & external capsules	☐	☐			☐	☐		
g.	Thalamus	☐	☐			☐	☐		
h.	Deep & periventricular white matter (including corpus callosum)	☐	☐			☐	☐		
	DEEP Total	☐	☐			☐	☐		
l.	Cerebellum	☐	☐	☐	☐	☐	☐	☐	☐
m.	Brainstem	☐	☐			☐	☐		
	INFRATENTORIAL Total	☐	☐	☐	☐	☐	☐	☐	☐

Comments

Version 2 21/04/17



Atrophy Ranking (75-80 Year)

CT diagnostic criteria for CAA-associated lobar ICH external validation protocol

1. Title

1.1. The Edinburgh CT and genetic diagnostic criteria for lobar intracerebral haemorrhage associated with cerebral amyloid angiopathy: external validation

2. Background

2.1. Non-invasive diagnosis of CAA in lobar ICH is important for research studies and to guide estimates of prognosis and some treatment decisions in clinical practice.[1-3]

2.2. The recently published Edinburgh diagnostic criteria for CAA-associated lobar ICH use diagnostic CT features (subarachnoid haemorrhage or finger-like projections from the haematoma) along with APOE ε4 genotype if available to predict the risk of moderate or severe CAA.[4] The diagnostic models showed excellent discrimination and good calibration in the development setting. The models were internally validated using bootstrapping, which showed very small optimism in the models (e.g. for the c statistic, the apparent performance = 0.92, while optimism adjusted performance = 0.91). This suggests the models are not over-fitted and confirms their reproducibility and validity in the development setting. However, the performance of the models need to be assessed in other cohorts to determine their accuracy and clinical utility in different settings.

2.3. The International CAA Association is currently coordinating an external validation and update of the modified Boston MRI criteria. This provides the ideal opportunity to perform a multi-centre external validation study of the Edinburgh CT and genetic diagnostic criteria. The goal is to present results from this collaborative project at the next International CAA conference (Lille, September 2018).

3. Aims

3.1. Perform external validation of the Edinburgh diagnostic prediction models for moderate/severe CAA associated with lobar intracerebral haemorrhage (ICH) (using the CT-only and CT + APOE diagnostic models separately) against a histopathological reference standard.

3.2. Assess inter-rater agreement for subarachnoid haemorrhage and finger-like projections on brain CT.

CT diagnostic criteria for CAA-associated lobar ICH external validation protocol

4. Study design

4.1. Study cohort(s)

4.1.1. Identified from existing collaborations within the international CAA association and other groups with suitable data available

4.2. Eligibility criteria

4.2.1. First-ever (i.e. not recurrent) lobar ICH

4.2.1.1. Diagnosed by non-contrast brain CT

4.2.1.2. Lobar ICH location:[5] definite lobar ICH (the main bulk and presumed epicentre of the haematoma is located in the cerebral cortex or at the junction of the cortex and white matter [including subcortical white matter], and does not extend into the subcortical grey matter structures), or probable lobar ICH (the ICH is difficult to distinguish visually between lobar and non-lobar origin, but the main bulk and presumed epicentre of the haematoma is located in the cerebral cortex or at the junction of the cortex and white matter [including subcortical white matter]), or multiple simultaneous ICHs in solely lobar locations.

4.2.1.3. No evidence of underlying cause (e.g. an underlying tumour, intracranial vascular malformation, venous thrombosis, prior trauma or haemorrhagic conversion of a cerebral infarct) other than cerebral small vessel disease.

4.2.2. Age ≥16 years at ICH

4.2.3. Index tests (diagnostic quality non-contrast brain CT ± APOE genotype) and reference standard (sufficient tissue for histopathological assessment of CAA) both available.

4.2.4. Not included in the Edinburgh LINCHPIN cohort (1st June 2010 to 10th February 2016)[4]

4.3. Index tests

4.3.1. First diagnostic non-contrast brain CT performed after first-ever ICH onset (compulsory), available in DICOM format. Desirable: volume data set to allow reformatting into standard planes. Minimum: axial plane acquisition.

4.3.2. APOE ε4 genotype (optional) from peripheral blood or brain tissue.

4.4. Reference standard

4.4.1. Histopathological evaluation for CAA by brain biopsy or autopsy (not haematoma evacuation), with histological staining plus amyloid-β immunohistochemistry performed for CAA evaluation.

CT diagnostic criteria for CAA-associated lobar ICH external validation protocol

5. Index and reference test acquisition and rating

5.1. Diagnostic non-contrast brain CT (compulsory)

5.1.1. Images will be reviewed in standard planes: axial (parallel to a line linking the floor of the sella turcica to the fastigium of the fourth ventricle); coronal (parallel to the posterior surface of the brain stem); sagittal (parallel to the interhemispheric fissure).

5.1.2. Ratings. Raters undergo CT imaging assessment training for finger-like projections and then rate CTs using a standardised proforma. Compulsory ratings: ICH location, presence of subarachnoid haemorrhage and finger-like projections. Desirable ratings: ICH volume (ABC/2 or other appropriate method), presence of intraventricular and subdural haemorrhage, CT features of small vessel disease (white matter lucencies, lacunes, atrophy). Raters will be masked to clinical, genetic and pathological data. Ratings will be performed centrally by at least one Edinburgh and one non-Edinburgh rater. Final ratings where there is disagreement between the two raters will be based on consensus.

5.2. APOE ε4 genotype (optional)

5.2.1. Genotypes for two APOE single-nucleotide polymorphisms (rs429358 and rs7412). APOE ε4 possession = presence of at least one ε4 allele (see highligted rows in below table).[6] Genotype calling should be masked to clinical, radiological and pathological data and performed locally by collaborator.

rs429358	rs7412	Genotype
C;C	T;T	Apo-ε1/ε1
C;T	T;T	Apo-ε1/ε2
C;T	C;T	Apo-ε2/ε4
C;C	C;T	Apo-ε1/ε4
T;T	T;T	Apo-ε2/ε2
T;T	C;T	Apo-ε2/ε3
T;T	C;C	Apo-ε3/ε3
C;T	C;C	Apo-ε3/ε4
C;C	C;C	Apo-ε4/ε4

5.3. Histopathological CAA evaluation (compulsory)

5.3.1. Assessed using Vonsattel scale.[7,8] Grade ≥2 (complete replacement of media by amyloid-β-positive material) for both biopsy and autopsy.

5.3.2. Masked to clinical, radiological and genetic data.

5.3.3. Performed either locally at source hospital by experienced neuropathologist or tissue slides provided for central review in Boston if local expertise not available

5.3.4. Provide pathology report, and representative images if available.

6. Patient characteristics to collect

6.1. Age at onset of first-ever ICH leading to study inclusion

6.2. Date of first-ever ICH symptom onset leading to study inclusion

6.3. Sex

6.4. Admission Glasgow Coma Scale (GCS) Score

6.5. Date of diagnostic brain CT

6.6. Date of cortical biopsy or death leading to acquisition of the tissue sample used as the reference standard

6.7. Comorbidities before ICH

6.7.1. Hypertension. Defined as either a history of hypertension in medical records before ICH or taking antihypertensive medication at the time of the ICH

6.7.2. Dementia. Defined as either a diagnosis of dementia in medical records before ICH or if a relative or close friend completed the short form Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) and the score was ≥64.[9]

6.7.3. Prior ischaemic stroke or TIA

6.7.4. Myocardial infarction

6.7.5. Atrial fibrillation

6.7.6. Diabetes mellitus

6.7.7. Hypercholesterolemia

6.7.8. Smoking

6.8. Medication taken at the time of ICH

6.8.1. Antiplatelet drug(s)

6.8.2. Anticoagulant drug(s)

CT diagnostic criteria for CAA-associated lobar ICH external validation protocol

7. Statistical analysis plan

7.1. This is based on TRIPOD[10] guidelines for prediction model studies, and literature for validating clinical prediction models.[11-14] Analyses will be conducted by Mark Rodrigues (Edinburgh University), and reproduced independently by a non-Edinburgh researcher.

7.2. Describe flow of participants including exclusions

7.3. Comparison of the characteristics of the development (Edinburgh) and external validation cohorts (this determines whether the reproducibility [similar case-mix] or transportability [different case-mix] is being assessed).

7.3.1. Demographics and baseline clinical features (age, sex, hypertension, pre-ICH dementia, pre-ICH anti-thrombotic use, admission GCS etc)

7.3.2. Time between symptom onset & CT, CT & tissue sample, survival after ICH

7.3.3. ICH details (location, volume, IVH, subdural extension)

7.3.4. Predictors: subarachnoid haemorrhage, finger-like projections, APOE ε4 possession

7.3.5. We may stratify these analyses by pathology source (biopsy versus autopsy) and those with/without APOE genotype

7.4. Diagnostic prediction model performance

7.4.1. Assess the performance of both CT-only and CT + APOE diagnostic models separately

7.4.2. Stratify by type of pathological reference standard (biopsy versus autopsy) and by age for biopsy samples (decreasing specificity for CAA with increasing age)

7.4.3. Use prediction models' regression equations to calculate predicted probabilities of CAA-associated lobar ICH. Compare against observed frequencies of moderate/severe CAA.

7.4.4. Overall performance: Brier score, R^2 (Nagelkerke), AIC

7.4.5. Calibration: calibration plot & Hosmer-Lemeshow test; calibration-in-the-large (intercept) and calibration slope

7.4.6. Assess discrimination using c statistic and ROC curve

7.4.7. Net benefit using decision curve analysis[15]

7.4.8. Assess classification measures (sensitivity, specificity, likelihood ratios, predictive values etc) of the published Edinburgh diagnostic criteria low, intermediate and high risk groups

7.5. Consider model updating if relevant

CT diagnostic criteria for CAA-associated lobar ICH external validation protocol

8. **Sample size**

   8.1. This should be as large as possible because general guidance indicates that we are likely to need at least 100 cases with CAA and 100 cases without CAA to detect modest changes in discrimination and calibration-in-the-large.[11,16]

   8.2. We will aim to maximise the number of cases with autopsy reference standard, but will include cases with cortical biopsy if required to reach our desired sample size.

9. **Missing data**

   9.1. We will conduct complete case analysis and we will not impute missing data.

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Reference List

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The University of Edinburgh
Edinburgh Neuroscience

CCBS
Centre for Clinical Brain Sciences

26 September 2019

Dear xxx

Invitation to join the International CAA Association (ICAAA) collaborative external validation of the Edinburgh diagnostic prediction models for cerebral amyloid angiopathy-associated with lobar intracerebral haemorrhage

We are writing in the hope that you will contribute to a multicentre external validation study of the Edinburgh diagnostic prediction models for cerebral amyloid angiopathy (CAA)-associated with lobar intracerebral haemorrhage (ICH). We intend to submit this study to a major medical journal by the autumn. In brief, the paper will include 1 or 2 authors from each group that contributes data, but we are very happy to discuss the details of collaboration. Other members of the contributing research groups will be listed as collaborators on PubMed (further details here).

Non-invasive diagnosis of CAA in lobar ICH is important for research studies and clinical practice. The recently published Edinburgh diagnostic criteria for CAA-associated lobar ICH use diagnostic CT features (subarachnoid haemorrhage and finger-like projections from the haematoma), along with APOE genotype (ε4 allele possession) if available, to predict the risk of moderate or severe CAA. However, the diagnostic performance of the criteria need to be assessed in other cohorts to determine their accuracy and clinical utility in different settings.

The International CAA Association is coordinating an external validation and update of the modified Boston MRI CAA criteria. This provides the ideal opportunity to perform a multicentre external validation study of the Edinburgh CT and genetic diagnostic criteria. Your group's work[1] *title* is one of the 15 or so series that we have identified by systematically reviewing the literature, in which patients with lobar ICH have undergone a diagnostic non-contrast brain CT scan and histopathological assessment for the presence or absence of CAA. We already have data for more than x patients from groups in Boston, USA and Edinburgh, UK, but we believe that the data from your study will be an important contribution to this collaborative study. Further details of the study are included in the accompanying summary protocol.

Please respond by sending us the attached form by xxxx. We will need you to provide clinical characteristics (including APOE genotype, if available) and DICOM files of the brain CT imaging by xxxx, when the imaging will be rated in Boston. We will be delighted to discuss any aspect of this proposal with you (contact details in the footer below).

With best wishes,

Dr Mark Rodrigues
Clinical Fellow
The University of Edinburgh

Dr Andreas Charidimou

[1] Insert

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Please complete and send as a PDF to mark.rodrigues@ed.ac.uk or fax to +44 (0)131 537 2944

Please ✓ one answer to each question:

1.	Would you like to co-author and share data with this study?	**Yes** ☐	**No** ☐
2.	Do you have cases meeting the following eligibility criteria?		
	a Essential • Participants with first-ever (i.e. not recurrent) lobar ICH diagnosed by non-contrast brain CT • Age ≥16 years at ICH onset • No evidence of underlying cause other than cerebral small vessel disease • First diagnostic non-contrast brain CT performed after first-ever ICH onset available in DICOM format • Histopathological evaluation for CAA by brain biopsy or autopsy (not haematoma evacuation)	**Yes** ☐	**No** ☐
	b Optional • APOE ε4 genotype	**Yes** ☐	**No** ☐
	c If yes, approximately how many cases with the following do you have? • Lobar ICH with CT ± APOE and histopathological evidence of CAA		
	• Lobar ICH with MRI, CT ± APOE and no histopathological evidence of CAA		
3.	If you have eligible cases, do you have the following details about them?		
	a. Sex	**Yes** ☐	**No** ☐
	b. History of hypertension before ICH	**Yes** ☐	**No** ☐
	c. History of dementia before ICH	**Yes** ☐	**No** ☐
	d. Date of first-ever ICH symptom onset	**Yes** ☐	**No** ☐
	e. Age at onset of first-ever ICH	**Yes** ☐	**No** ☐
	f. Antiplatelet drug(s) use at the time of ICH	**Yes** ☐	**No** ☐
	g. Anticoagulant drug(s) use at the time of ICH	**Yes** ☐	**No** ☐
	h. Admission Glasgow Coma Scale (GCS) score	**Yes** ☐	**No** ☐
	i. Date of diagnostic brain CT	**Yes** ☐	**No** ☐
	j. Date of cortical biopsy or autopsy	**Yes** ☐	**No** ☐

The association between the Edinburgh CAA criteria and recurrent ICH risk

1. Title

1.1. Association between the Edinburgh CT and genetic diagnostic criteria for lobar intracerebral haemorrhage (ICH) associated with cerebral amyloid angiopathy and the risk of recurrent ICH: outline protocol for a population-based analysis, and external validation in a hospital-based cohort

2. Background

2.1. Lobar intracerebral haemorrhage (ICH) associated with MRI biomarkers of cerebral amyloid angiopathy (CAA) seems to have a higher risk of recurrent ICH than other sub-types of ICH, and may increase the risk of antithrombotic-related ICH. [1,2]

2.2. Non-invasive diagnosis of CAA in lobar ICH is therefore important to estimate prognosis and guide some treatment decisions in clinical practice.[1,2]

2.3. The principal approach for non-invasive diagnosis of CAA uses the MRI-based modified Boston criteria.[3] However MRI may be contraindicated, inappropriate in the acute setting, or unavailable (particularly in low and middle-income countries).

2.4. The recently published Edinburgh diagnostic criteria for CAA-associated lobar ICH use two diagnostic CT features (subarachnoid haemorrhage and finger-like projections from the haematoma) along with APOE ε4 genotype (if available) to predict the risk of moderate or severe CAA.[4] In the development setting, the rule-out and rule-in diagnostic criteria showed excellent sensitivity and specificity for moderate or severe CAA respectively, which were best when using both diagnostic CT features and APOE ε4 genotype.

2.5. The association between the Edinburgh diagnostic criteria and recurrent ICH risk is unknown.

3. Aim

3.1. Investigate the association between the Edinburgh diagnostic criteria (both CT-only and CT+APOE criteria separately)[4] with risk of recurrent ICH in survivors of lobar ICH.

4. Study design

4.1. Study cohorts

4.1.1. Internal cohorts

- Lothian audit of the treatment cerebral haemorrhage (LATCH)

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3-year (2010-2013), population-based, prospective inception cohort audit of spontaneous ICH in the Lothian health board region of Scotland

- Lothian intracerebral haemorrhage, pathology, imaging and neurological outcome (LINCHPIN) study
  7-year (2010-2017) community-based inception cohort study of spontaneous ICH in the Lothian health board region of Scotland

4.1.2. External cohorts

- Primary external validation cohort: CROMIS-2 ICH cohort study
  Multicentre, prospective cohort study of spontaneous ICH
- Other cohorts that might be suitable for external validation have spontaneously expressed interest, and may contribute to future external validation studies of the associations being explored in the internal cohorts and the primary external validation cohort

4.2. Eligibility criteria

4.2.1. First-ever or recurrent ICH

- Diagnosed by non-contrast brain CT
- Lobar ICH location:[5] definite lobar ICH (the main bulk and presumed epicentre of the haematoma is located in the cerebral cortex or at the junction of the cortex and white matter [including subcortical white matter], and does not extend into the subcortical grey matter structures), or probable lobar ICH (the ICH is difficult to distinguish visually between lobar and non-lobar origin, but the main bulk and presumed epicentre of the haematoma is located in the cerebral cortex or at the junction of the cortex and white matter [including subcortical white matter]), or multiple simultaneous acute ICHs in solely lobar locations.
- No evidence of underlying cause (e.g. an underlying tumour, intracranial vascular malformation, venous thrombosis, prior trauma or haemorrhagic conversion of a cerebral infarct) other than cerebral small vessel disease.

4.2.2. Age ≥16 years at ICH onset

4.2.3. Diagnostic non-contrast brain CT available for analysis

4.2.4. APOE ε4 genotype (optional) from peripheral blood or brain tissue.

5. Patient characteristics to collect

5.1. Age at onset of index ICH leading to study inclusion

5.2. Sex

5.3. Date of index ICH symptom onset

5.4. Admission Glasgow Coma Scale (GCS) Score

5.5. Comorbidities before ICH

- Hypertension. Defined as either a history of hypertension in medical records before ICH or taking antihypertensive medication at the time of the ICH
- Dementia. Defined as either a diagnosis of dementia in medical records before ICH or if a relative or close friend completed the short form Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) and the score was ≥64.[6]
- Prior ischaemic stroke or TIA
- Myocardial infarction
- Atrial fibrillation
- Diabetes mellitus
- Hypercholesterolemia
- Smoking

5.6. Medication taken at the time of ICH

- Antiplatelet drug(s)
- Anticoagulant drug(s)
- Anti-hypertensive drug(s)

5.7. Medication at time of discharge

- Antiplatelet drug(s)
- Anticoagulant drug(s)
- Anti-hypertensive drug(s)

6. Image analysis

6.1. Diagnostic non-contrast brain CT

6.1.1. Ideally, reformat volume data sets into standard planes:[4] axial (parallel to a line linking the floor of the sella turcica to the fastigium of the fourth ventricle); coronal (parallel to the posterior surface of the brain stem); sagittal (parallel to the interhemispheric fissure); standard slice thickness (5mm), spacing (3mm), and windowing (centre 35, width 80).

6.1.2. Ratings.

6.1.2.1. Performed either in Edinburgh or at the coordinating centre for external validation cohort

6.1.2.2. Raters masked to clinical, genetic and outcome data.

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6.1.2.3. Baseline CT features

- ICH location determined according to published criteria[5]
- ICH volume (ABC/2 or other appropriate method)
- Presence of IVH, subdural haemorrhage

6.1.2.4. Edinburgh CT criteria

- Fingerlike-projections:[4] elongated extension arising from the haematoma, longer than they are wide regardless whether they extend to the cortex or not.
- Subarachnoid haemorrhage: haemorrhage in the extra-axial subarachnoid space.

6.1.2.5. CT features of small vessel disease

- White mater lucencies rated with the Van Swieten Scale.[7] Combine the posterior (range 0-2) and anterior (range 0-2) scores into a 5-point ordinal scale (0-4)
- Presence and number of lacunes defined as round or ovoid-shaped small CSF attenuation areas <1.5 cm in diameter in subcortical white and deep grey matter.[8]
- Atrophy defined as deep or cortical, and rated with a 3-point ordinal scale as none, moderate, or severe against a reference CT brain template.[9]

6.1.2.6. CT SVD score[10] - 1 point for each of the following if present:

- Severe lucencies (Van Swieten Scale = 2) in anterior or posterior periventricular white matter
- Lacunes ≥2
- Severe (=2) central or cortical atrophy

The combined 4-point ordinal score therefore assesses the global burden of SVD from 0 (no imaging features of severe SVD) to 3 (imaging features of SVD scored as severe for each imaging variable)

6.2. APOE ε4 genotype (optional)

6.2.1. Genotypes for two APOE single-nucleotide polymorphisms (rs429358 and rs7412). APOE ε4 possession = presence of at least one ε4 allele (see highlighted rows in below table).[11] Genotype calling should be masked to clinical, radiological and outcome data.

rs429358	rs7412	Genotype
C;C	T;T	Apo-ε1/ε1
C;T	T;T	Apo-ε1/ε2
C;T	C;T	Apo-ε2/ε4
C;C	C;T	Apo-ε1/ε4
T;T	T;T	Apo-ε2/ε2
T;T	C;T	Apo-ε2/ε3
T;T	C;C	Apo-ε3/ε3
C;T	C;C	Apo-ε3/ε4
C;C	C;C	Apo-ε4/ε4

7. Follow up and outcome measures

7.1. Follow up

7.1.1. Comprehensive clinical follow-up for recurrent ICH and death by telephone interview, in-person assessment, patient and GP postal questionnaires, hospital electronic patient records, or secondary data sources (e.g. registries of death certificates and hospital attendances, radiology information systems etc)

7.1.2. Review of all relevant brain imaging (or reports) required to confirm recurrent ICH

7.2. Outcome of interest: first recurrent ICH

7.2.1. Defined as onset of new neurological deficits or worsening of pre-existing deficits, anatomically referable to evidence of new ICH on CT or MRI scans

7.2.2. Adjudicated by trained member using all available clinical and imaging information, masked to baseline clinical and imaging data

7.3. Competing event: death

8. Statistical analysis plan

8.1. Analysis to be performed by Mark Rodrigues (University of Edinburgh) using R statistical package

- Dataset and code can be made available to enable replication of results

8.2. Describe flow of participants through different cohorts, including exclusions

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8.3. Description of the cohorts

- Demographics and baseline clinical features (age, sex, hypertension, pre-ICH dementia etc)
- Medications on admission (anti-platelet, anti-coagulant, antihypertensives)
- Admission GCS
- ICH details (location, volume, IVH, subdural extension)
- Edinburgh diagnostic criteria: subarachnoid haemorrhage, finger-like projections, APOE ε4 possession
- Medications on discharge (anti-platelet, anti-coagulant, antihypertensives)

8.4. Inter-rater agreement of CT features (if available)

8.5. Completeness of follow up of the cohorts

8.5.1. Completeness index[12] ([observed follow up/potential follow up]*100)

8.6. Time to event analysis

8.6.1. Outcome = 1st recurrent ICH; competing event = death

- NB Methods of standard survival analysis such as the Kaplan-Meier method for estimation of cumulative incidence, the log-rank test for comparison of cumulative incidence curves, and the standard Cox model for the assessment of covariates lead to incorrect and biased results in the presence of competing events

8.6.2. Performed in the internal and external cohorts separately, then meta-analysis of both cohorts to increase power

- Edinburgh CT-only criteria (LATCH & external cohorts)
- Edinburgh CT & genetic criteria (LINCHPIN & external cohorts)

8.6.3. Primary analysis:

8.6.3.1. Restrict to those with 1st-ever ICH as index event surviving at least 30 days (the focus is on recurrent ICH risk in those likely to survive to hospital discharge, to minimise the impact of the high early competing risk of death)

8.6.3.2. Cumulative incidence function of recurrent ICH in the presence of competing event (death)

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- Edinburgh CAA diagnostic categories (low vs int vs high; low vs int/high)
- Sex
- CT SVD score (0 vs 1,2 or 3)

8.6.3.3. Competing risks multivariable regression

- Edinburgh CAA diagnostic criteria low (reference) vs int vs high
    - Should there be insufficient power to examine all three Edinburgh CAA diagnostic strategies we will examine low vs int/high
- Adjust for age, sex, CT SVD score (0 vs some) & APOE ε2 allele possession (based on ~40 events)
- time point for analysis will depend on confirmation of proportional hazards

8.6.4. Secondary analysis:

8.6.4.1. Include those with recurrent ICH as index event

8.6.4.2. Other candidate variables for univariate and multivariable

- Baseline hypertension, baseline dementia
- Recurrent ICH as index event
- Antithrombotic and antihypertensive drug use at discharge
- If Edinburgh criteria associated with recurrent ICH, we will conduct exploratory analyses of the individual components and APOE ε2 allele possession
- Number of variables will depend on the number of outcomes (7-10 events/variable) and time point for analysis will depend on confirmation of proportional hazards

8.6.5. Confounders

8.6.5.1. Hypertension and antithrombotic drug use

- These are difficult to treat as time depending covariates due to data collection and competing risk analysis methods. Therefore, we will take a descriptive approach
    - Describe antithrombotic drug use at hospital discharge or day 30 after ICH
    - Describe anti-hypertensive use at hospital discharge or day 30 after ICH

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- Describe blood pressure during follow up in key groups, such as people with the outcome (recurrent ICH) vs people without outcomes and Edinburgh CAA criteria groups
- Describe BP as both a continuous variable (mean or median as appropriate) and categorical (systolic BP ≤130mmHg vs >130mmHg)

9. Missing data

9.1. We will conduct complete case analysis for predictor and outcome variables. We will not impute missing data.

The association between the Edinburgh CAA criteria and recurrent ICH risk

Reference List

1. Charidimou A, Imaizumi T, Moulin S, et al. Brain hemorrhage recurrence, small vessel disease type, and cerebral microbleeds: A meta-analysis. *Neurology* 2017; **89**(8): 820-9.
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